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(54) Title: PHOSPHOLIPASE INHIBITOR (57) Abstract <p>The present invention relates generally to a broad-spectrum phospholipase enzyme inhibitor and uses therefor. More particularly, the present invention provides an inhibitor of phospholipase A₂ enzymes, wherein the inhibitor is a proteinaceous molecule including a peptide, polypeptide or protein which is derivable from the serum of a venomous animal. The present invention extends to derivatives, homologues, analogues, mimetics and functional chemical equivalents of the phospholipase A₂ inhibitor. The phospholipase A₂ inhibitor of the present invention is particularly useful in the production of a wide range of human and veterinary pharmaceutical products such as for the treatment of conditions involving phospholipase A₂ including, but not limited to, rheumatoid arthritis, osteoarthritis, asthma, allergic conditions, psoriasis, autoimmune disorders, inflammatory disease, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome, insect and snake bite, amongst others.</p>		

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PHOSPHOLIPASE INHIBITOR

FIELD OF INVENTION

- 5 The present invention relates generally to a broad-spectrum phospholipase enzyme inhibitor and uses therefor. More particularly, the present invention provides an inhibitor of phospholipase A₂ enzymes, wherein the inhibitor is a proteinaceous molecule including a peptide, polypeptide or protein which is derivable from the serum of a venomous animal. The present invention extends to derivatives, homologues, analogues, mimetics and
- 10 functional chemical equivalents of the phospholipase A₂ inhibitor. The phospholipase A₂ inhibitor of the present invention is particularly useful in the production of a wide range of human and veterinary pharmaceutical products such as for the treatment of conditions involving phospholipase A₂ including, but not limited to, rheumatoid arthritis, osteoarthritis, asthma, allergic conditions, psoriasis, autoimmune disorders, inflammatory disease, multiple
- 15 organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome, insect and snake bite, amongst others.

BACKGROUND OF INVENTION

- 20 Bibliographic details of publications referred to by author in this specification are collected at end of the description.

Phospholipase A₂ (PLA₂) is a carboxylic acid esterase which removes the unsaturated fatty acid at the C-2 of glycerol.

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PLA₂ enzymes comprise several sub-types including human Type I PLA₂, which is derived from human pancreas (Dennis, 1994; Dennis, 1997) and human Type II PLA₂ which is derived from human synovium. A Type III PLA₂ also exists.

- 30 Known PLA₂ enzymes are extremely stable to high temperatures and treatment with denaturing agents such as diethyl ether, chloroform or 8M urea, presumably due to their

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compact structures.

Phospholipases, and in particular PLA_2 , produce several adverse effects in humans and animals when administered, for example, in a venom, or when over produced by the body itself. For example, PLA_2 leads to the production of arachidonic acid which in turn may form arachidonic acid metabolites having proinflammatory activity (Flower *et al.*, 1979). Additionally, the PLA_2 in bee and snake venom is largely responsible for their toxicity to humans and animals.

- 10 Although many PLA_2 inhibitors are known, only a few proteinaceous PLA_2 inhibitors have been described. For example, a 344 amino acid protein has been obtained from the serum of mammals by enzymatic treatment. This molecule is useful in the treatment and diagnosis of inflammatory disease (United States Patent No. 5,344,764). Most PLA_2 inhibitors are synthetic chemical compounds possessing highly specific anti-inflammatory activity (e.g.
- 15 ARL-67974, Astra; picolinic acid derivatives; thio-tetronic acid derivatives; 4-phenylakenoic and alkienoic acid PLA_2 inhibitors) or which prevent multiorgan failure.

- Notwithstanding availability of some PLA_2 inhibitors, until advent of the present invention, all known PLA_2 inhibitors, including proteinaceous inhibitors, chemical compounds and
- 20 small molecules, are limited in their range of inhibition. PLA_2 inhibitors which have previously been derived from snake venom, before instant invention, have also had a limited range of inhibition and are capable of inhibiting only snake PLA_2 enzymes. There is a need to identify PLA_2 inhibitors of general utility in the inhibition of PLA_2 enzyme activities.

- 25 In work leading up to the present invention, the inventors identified factors in the serum of snakes which protect the snakes against the toxic effects of their own venom or the venom of other animals. It has been surprisingly shown by the inventors that the main protective factor present in serum of snakes was capable of generally inhibiting PLA_2 enzymes. The purified PLA_2 inhibitor of the present invention provides a means for producing a wide range of
- 30 pharmaceutical compounds of utility in the treatment of inflammatory conditions, autoinflammatory conditions, multiple organ failure, acute pancreatitis, acute lung failure,

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septic shock, adult respiratory stress and the toxic effects of PLA₂ enzymes in insect and snake venoms, amongst other uses.

SUMMARY OF THE INVENTION

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Abbreviations for phospholipase inhibitors are summarized in Table 1. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of the SEQ ID NOs. is given in Table 2.

10

Throughout this specification, the term "at least" will be understood to mean that a stated integer or group of integers performs a stated function or is included in a stated composition of matter, but not to the exclusion of other functions or integers or groups of integers.

15 Throughout this specification, unless context requires otherwise, word "comprise", or variations such as "comprises", "at least comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

20 One aspect, an isolated molecule which is capable of inhibiting two or more phospholipase enzymes.

Another aspect of the present invention is directed to an isolated peptide, polypeptide or protein or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical

25 equivalent thereof which is capable of inhibiting two or more PLA₂ enzymes.

Yet another aspect of the present invention contemplates an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis scutatus* and is capable of inhibiting two or more of PLA₂ Type I, II

30 and/or III enzymes. This molecule is referred to herein as "NSI".

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Even yet another aspect of the present invention provides an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis ater* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "NAI".

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Still yet another aspect of the present invention contemplates an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus scutellatus* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "OSI".

10

Even yet another aspect of the present invention provides an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus microlepidotus* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "OMI".

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Another aspect of the present invention relates to an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Pseudonaja textilis* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "PTI".

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A further aspect of the present invention provides a PLA₂ inhibitor having a β -chain comprising an amino acid sequence substantially as set forth in one of SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 or 87 or an amino acid sequence having at least 40% similarity to one or more of the above listed sequences.

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Yet a further aspect of the present invention contemplates a PLA₂ inhibitor having a β -chain encoded by a nucleotide sequence comprising a sequence as set forth in one of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence
30 capable of hybridizing under low stringency conditions at 42°C to one or more of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90.

- 5 -

Still yet another aspect of the present invention is directed to an isolated PLA₂ inhibitor comprising an α -chain comprising an amino acid sequence set forth in one of SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 or 85 or an amino acid sequence having at least about 40% similarity to one or more of the above listed sequences.

5

Another aspect of the present invention is directed to a PLA₂ inhibitor comprising an α - and β -chain wherein the α -chain comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of the above sequence and a β -chain comprising an amino acid sequence selected from SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least 40% similarity to one or more of the latter sequences.

A further aspect of the present invention provides a PLA₂ inhibitor comprising α - and/or β -chains having amino acid sequences or encoded by nucleotide sequence substantially as set forth for NSI, NAI, OSI, OMI and PTI in Table 2.

Accordingly, another aspect of the present invention contemplates a PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof comprising structure:



wherein

25 α is an α -chain of a PLA₂ inhibitor;

β is a β -chain of a PLA₂ inhibitor;

m is an integer from 0 to 10;

n is an integer from 0 to 10

with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid

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sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences. Preferably, m is 2-4 and n is 1-2. More
5 preferably, m is 2 and n is 1.

A further aspect of the present invention contemplates a composition comprising an isolated or recombinant phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof together with one or more pharmaceutically acceptable carriers
10 and/or diluents.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a phospholipase inhibitor or a homologue or derivative of said phospholipase
15 inhibitor.

Another aspect of the present invention provides an isolated nucleic acid molecule having an α -chain encoded by a nucleotide sequence comprising as set forth in one of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence
20 having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing to one or more of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 under low stringency conditions at 42°C.

Yet another aspect of the present invention provides an isolated nucleic acid molecule having
25 a sequence of nucleotides or complementary sequence of nucleotides comprising one or more of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82 to 84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to any one or more of said sequences under low stringency conditions at 42°C.

30

Yet another aspect of the present invention provides a nucleic acid molecule which encodes

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an α -chain polypeptide of a PLA₂ inhibitor protein or a homologue or derivative thereof which nucleotide sequence has at least about 75% similarity to one or more of the nucleotide sequences set forth in SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54-55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence capable of hybridizing under low stringency conditions at 5 42°C to one or more of said sequence.

Still yet another aspect of the present invention contemplates an isolated nucleic acid molecule which encodes a β -chain polypeptide or a PLA₂ inhibitor protein or a homologue or derivative thereof which has at least about 75% similarity to any one of the nucleotide 10 sequences set forth in SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence capable of hybridising under at least low stringency conditions at 42°C to one of said sequences.

Even yet another aspect of the present invention provides a nucleic acid molecule encoding a 15 PLA₂ inhibitor having the structure:



wherein

- 20 α is an α -chain of a PLA₂ inhibitor;
 β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;
 n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 25 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

- 30 Still even yet another aspect of the present invention provides a nucleic acid molecule encoding a PLA₂ inhibitor having the structure:

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wherein

α is an α -chain of a PLA₂ inhibitor;

5 β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by a nucleotide sequence selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and 88 or a nucleotide sequence having at least about 40% similarity to one or more of said
10 sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1a is a graphical representation showing the elution profile for *N. scutatus* serum from A DEAE-Sephacel column. The SPP containing NSI elutes in P4.

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Figure 1b is a graphical representation showing the further purification of SPP on a Mono-S column.

Figure 2a is a graphical representation showing the inhibition of snake venom PLA₂ enzymes
25 with NSI, Day 1.

Figure 2b is a graphical representation showing the inhibition of snake venom PLA₂ enzymes with NSI, Day 2.

30 **Figure 3a** is a graphical representation showing the inhibition of non-snake venom PLA₂ by NSI, dilution group 1.

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Figure 3b is a graphical representation showing the inhibition of non-snake venom PLA₂'s by NSI, dilution group 2.

Figure 4 is a graphical representation showing the inhibition of rhPLA₂ by NSI.

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Figure 5a is a graphical representation showing the pH stability of NSI.

Figure 5b is a graphical representation showing the temperature stability of NSI.

10 Figure 6a is a graphical representation showing the inhibition by deglycosylated NSI.

Figure 6b is a graphical representation showing the elution pattern for native (NSI) and deglycosylated NSI (DGNSI) from a size exclusion column. NSI elutes at 21-27 minutes.

15 Figure 7 is a graphical representation showing the Superose 12 elution profiles of SPP, notexin and SPP: notexin complex.

Figure 8 is a graphical representation showing the purification of endoproteinase Glu-C digestion products of the α -chain of NSI.

20

Figure 9 is a graphical representation showing the purification of trypsin digestion products of the α -chain of NSI.

Figure 10 is a graphical representation showing the purification of β -chain tryptic peptides.

25 Peptides are marked T1 to T12 inclusive.

Figure 11 is a schematic representation showing the aligned amino acid sequences of phospholipase inhibitory α -chain polypeptides derived from *Notechis scutatus* (proseqns1; top row), coastal taipan *Oxyuranus scutellatus* (pseqct1; middle row) and inland taipan
30 *Oxyuranus microlepidotus* (pseqit1; lower row). Numbers indicate the amino acid position in each sequence.

TABLE 1

ABBREVIATIONS		
5	NSI	PLA ₂ inhibitor from <i>Notechis scutatus</i>
	NSI α i	α -chain from isoform i of NSI
	NSI α ii	α -chain from isoform ii of NSI
	NSI α iv	α -chain from isoform iv of NSI
	NSI β	β -chain from NSI
10	NSI α iL	leader sequence of NSI α i
	NSI α iiL	leader sequence of NSI α ii
	NSI α ivL	leader sequence of NSI α iv
	NSI β	leader sequence of NSI β
	NAI	PLA ₂ inhibitor from <i>Notechis ater</i>
15	NAI α i	α -chain from isoform i of NSI
	NAI α ii	α -chain from isoform ii or NSI
	NAI α v	α -chain from isoform v of NSI
	NAI β	β -chain from NAI
	NAI α iL	leader sequence of NAI α i
20	NAI α iiL	leader sequence of NAI α ii
	NAI α vL	leader sequence of NAI α v
	NAI β L	leader sequence of NAI β
	OSI	PLA ₂ inhibitor from <i>Oxyuranus scutellatus</i>
	OSI α i	α -chain from isoform i of OSI
25	OSI α ii	α -chain from isoform ii of OSI
	OSI β	β -chain from OSI
	OSI α iL	leader sequence of OSI α i

TABLE 1 (Continued)

	OSI α iiL	leader sequence of OSI α ii
	OSI β	leader sequence of OSI β
5	OMI	PLA ₂ inhibitor from <i>Oxyuranus microlepidotus</i>
	OMI α i	α -chain of isoform i of OMI
	OMI α ii	α -chain of isoform ii of OMI
	OMI β i	β -chain of isoform i of OMI
	OMI β ii	β -chain of isoform ii of OMI
10	OMI α iL	leader sequence of OMI α i
	OMI α iiL	leader sequence of OMI α ii
	OMI β iL	leader sequence of OMI β i
	OMI β iiL	leader sequence of OMI β ii
	PTI	PLA ₂ inhibitor from <i>Pseudonaja textilis</i>
15	PTI α ii	α -chain of isoform ii of PTI
	PTI β i	β -chain of isoform i of PTI
	PTI β ii	β -chain of isoform ii of PTI
	PTI α iL	leader sequence of PTI α i
	PTI β iL	leader sequence of PTI β i
20	PTI β iiL	leader sequence of PTI β ii

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TABLE 2
SUMMARY OF SEQ ID NOS.

5	Description	SEQ ID NOS.	
		Amino Acid Sequences	Nucleotide Sequences
	NSI α i	1	5
	NSI α ii	2	6
	NSI α iv	3	7
10	NSI β	4	8
	NSI α iL	9	13
	NSI α iiL	10	14
	NSI α ivL	11	15
	NSI β L	12	16
15	NAI α iN	17	-
	NAI α iiN	18	-
	NAI α ed	19-23	-
	NAI β N	24	-
	NAI β ed	25-34	-
20	NAI α i	35	39
	NAI α ii	36	40
	NAI α v	37	41
	NAI β	38	42
	NAI α iL	43	47
25	NAI α iiL	44	48
	NAI α vL	45	49
	NAI β L	46	50
	OSI α i	51	54

TABLE 2 (continued)

	OSI α ii	52	55
	OSI β	53	56
5	OSI α iL	57	60
	OSI α iiL	58	61
	OSI β L	59	62
	OMI α i	63	67
	OMI α ii	64	68
10	OMI β i	65	69
	OMI β ii	66	70
	OMI α iL	71	75
	OMI α iiL	72	76
	OMI β iL	73	77
15	OMI β iiL	74	78
	PTI α ii	79	82
	PTI β i	80	83
	PTI β ii	81	84
	PTI α iiL	85	88
20	PTI β iL	86	89
	PTI β iiL	87	90
	Oligonucleotide primers	91	
		94	

25 Abbreviations for Table 2

NSI, PLA₂ inhibitor from *Notechis scutatus*NAI, PLA₂ inhibitor from *Notechis ater*OSI, PLA₂ inhibitor from *Oxyuranus scutellatus*OMI, PLA₂ inhibitor from *Oxyuranus microlepidotus*30 PTI, PLA₂ inhibitor from *Pseudonaja textilis*

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α , α -chain of PLA₂ inhibitor
 β , β -chain of PLA₂ inhibitor
i, ii, iv, v, indicates number of isoform
L, Leader sequence
 α L, Leader sequence of α -chain
 β L, Leader sequence of β -chain
 β N, N-terminal region of β -chain
ed, Enzymatic digest

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides in one aspect, an isolated molecule which is capable of inhibiting two or more phospholipase enzymes.

5

The term "isolated" means that the molecule of the present invention is provided in a form which is distinct from that which occurs in nature, preferably wherein one or more contaminants have been removed. Accordingly, the isolated phospholipase inhibitor of the present invention may be partially-purified or substantially pure in which a substantial amount
10 of the contaminants have been removed or the inhibitor may be in sequencably pure or substantially homogeneous form.

The term "sequencably pure" means that the isolated phospholipase inhibitor is provided in a form which is sufficiently purified to facilitate amino acid sequence determination using
15 procedures known to those skilled in art.

The term "substantially homogeneous" includes an isolated phospholipase inhibitor of the present invention which is at least about 80% free of contaminants, more preferably at least about 90% free of contaminants, including 95-100% purity.

20

The preferred molecule of the present invention is a proteinaceous molecule such as but not limited to a peptide, polypeptide or protein. The present invention extends to recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms of the molecule and all such forms of the molecule are encompassed by use herein of term
25 "molecule" or "phospholipase inhibitor" or its abbreviation.

Preferably, the phospholipase is PLA_2 .

Accordingly, in a preferred embodiment, there is provided an isolated peptide, polypeptide or
30 protein or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof which is capable of inhibiting two or more PLA_2 enzymes. The peptide,

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polypeptide or protein, recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms of the PLA₂ inhibitor are all encompassed and included by use of the term "PLA₂ inhibitor" insofar as this term means the inhibitor of the present invention.

5 As exemplified herein, the present inventors have isolated and purified a PLA₂ inhibitor from serum of *Notechis scutatus*, *Notechis ater*, *Oxyuranus scutellatus*, *Oxyuranus microlepidotus* and *Pseudonaja textilis* by fractionation using anion exchange chromatography of dialysed serum and/or cation exchange chromatography. The present invention extends, however, to homologous PLA₂ inhibitors from other snakes and venomous
10 animals, such as those snakes and animals listed below.

By "inhibiting" is meant that the enzyme activity of a phospholipase enzyme is reduced in the presence of the PLA₂ inhibitor of the present invention, compared to the activity of the phospholipase enzyme in the absence of the inhibitor.

15

Accordingly, an "inhibitor" or "inhibitory substance" is a substance, and in particular a peptide, polypeptide or protein, which is capable of inhibiting phospholipase enzyme activity.

The term "phospholipase inhibitor" or "PLA₂ inhibitor" or similar term shall be taken to refer
20 to a peptide, polypeptide or protein or aggregates thereof such as a dimer or other multimer, fusion molecules or a homologue, analogue, mimetic, derivative or chemical equivalent thereof which is capable of inhibiting catalytic activity of a phospholipase enzyme, such as PLA₂ enzyme and in particular two or more PLA₂ enzymes.

25 For present purposes, a "phospholipase inhibitor" or "PLA₂ inhibitor" or similar term shall also be taken to include any peptide fragments or parts derived from a peptide, polypeptide or protein, aggregate or fusion molecules thereof or homologues, analogues, mimetics or chemical equivalents thereof, which, although they may have no inhibitory activity are at least useful as, for example, markers for antibody production, diagnostic markers, antagonists or
30 for other embodiments herein described.

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A "PLA₂ inhibitor" or "PLA₂ inhibitory protein" shall be taken to refer to a phospholipase inhibitor as hereinbefore defined which at least inhibits a PLA₂ enzyme but preferably more than one type of PLA₂ enzyme.

- 5 By "type of phospholipase [or PLA₂] enzyme" is meant a specific phospholipase such as a PLA₁, PLA₂, PLB, PLC or PLD enzyme. Preferably, it means more than one isoform of enzyme, for example, and in a most preferred embodiment, the Type I, Type II or Type III PLA₂.
- 10 In this regard, the inhibitory activity of the molecule of the present invention may be determined according to any standard method known to those skilled in art, such as by assaying for phospholipase enzyme activity in presence of the inhibitor as herein described or exemplified.
- 15 Those skilled in art will be aware that the amount of phospholipase inhibitor which is required to achieve inhibition may vary depending upon phospholipase enzyme being inhibited and/or the presence of other substances which may interfere with phospholipase activity inhibitor function.
- 20 In a preferred embodiment of invention, PLA₂ inhibitor described herein is capable of inhibiting at least 20%, more preferably at least about 50-70% and even more preferably at least about 80% of the PLA₂ activity present in a biological sample such as venom, synovial membrane, pancreas, skin, lung or other tissue or in association with an autoimmune response or inflammatory response such as an allergic reaction, rheumatoid arthritis, osteoarthritis,
- 25 asthma, psoriasis, acute pancreatitis, multiple organ failure, acute lung failure, septic shock or adult respiratory distress syndrome, amongst others.

In particular, the phospholipase inhibitors of the present invention exemplified herein have been shown by the inventors to inhibit all groups of PLA₂ enzymes against which it has been

30 tested. Additionally, the PLA₂ inhibitors of the present invention are capable of forming stable complexes with notexin (a purified PLA₂ enzyme) as judged by elution from a size

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exclusion column and also prevents radioiodinated notexin from binding to isolated rat brain synaptosomes. The significance of these novel features is that these PLA₂ inhibitors can be used to treat many different conditions where PLA₂ enzymes are implicated or known to act.

- 5 As stated above, the preferred phospholipase enzyme which is inhibited by the inhibitor of the present invention is phospholipase A₂ (PLA₂). More preferably, the PLA₂ enzyme is a Type I, II or III phospholipase PLA₂ enzyme.

Accordingly, another aspect of the present invention provides an isolated PLA₂ inhibitor, as
10 hereinbefore defined, capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes.

In one embodiment of the invention, the PLA₂ inhibitor is derived from the serum of an animal such as a snake or other reptile which produces a venom having toxic PLA₂ activity in
15 humans or other animals.

The term "derived from" shall be taken to refer to the origin of an integer or group of integers from a specified source, but not to the exclusion of another possible source or sources of the integer or group of integers.

20

In a particularly preferred embodiment of invention, the PLA₂ inhibitor is derived from a snake.

In a preferred embodiment, the present invention provides an isolated PLA₂ inhibitor or a
25 recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis scutatus* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "NSI".

In another preferred embodiment, the present invention provides an isolated PLA₂ inhibitor
30 or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis ater* and is capable of inhibiting two or more of PLA₂ Type I, II and/or

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III enzymes. This molecule is referred to herein as "NAI".

In yet another embodiment, the present invention provides an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent
5 thereof from *Oxyuranus scutellatus* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "OSI".

In still yet another aspect, the present invention provides an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent
10 thereof from *Oxyuranus microlepidotus* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "OMI".

Yet a further aspect of the present invention provides an isolated PLA₂ inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent
15 thereof from *Pseudonaja textilis* and is capable of inhibiting two or more of PLA₂ Type I, II and/or III enzymes. This molecule is referred to herein as "PTI".

The present inventors have determined that NSI is composed of two polypeptide chains, one 30 kDa glycosylated chain (α -chain) and one 25 kDa non-glycosylated chain (β -chain), as
20 determined using denaturing SDS/polyacrylamide gel electrophoresis (SDS/PAGE). By mass spectrometry, the estimated molecular weights of the α -chain and β -chain are 22,604 and 19,817 Da, respectively. The α -chain exists in a number of isoforms (at least 3) that vary in amino acid sequence and/or glycosylation pattern. These chains combine in a 2:1 ratio to form the intact NSI complex that has a molecular weight of 110 kDa as judged by size
25 exclusion chromatography. The glycosylation of the α -chain does not affect inhibition of PLA₂ enzymes. Similar ratios and polypeptide structures apply in relation to NAI, OSI, OMI and PTI.

The present invention clearly extends to all such isoforms of NSI, NAI, OSI, OMI and PTI
30 and reference to these molecules includes reference to all isoforms and their recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms.

- 20 -

The present invention extends further to a PLA₂ inhibitor which is capable of inhibiting a phospholipase enzyme wherein said molecule is capable of binding to the active site of a PLA₂ enzyme.

- 5 In a particularly preferred embodiment, the molecules according to this embodiment are capable of forming an interactive site with a phospholipase enzyme to inhibit the activity of enzyme. A "PLA₂ enzyme" includes one or more of PLA₂ Type I, II and/or III.

As used herein, the term "interactive site" shall be taken to refer to the primary, secondary or
10 tertiary structure of a phospholipase inhibitor of the present invention which is in physical relation with a phospholipase enzyme wherein said physical relation is required for inhibitory activity of said inhibitor, or at least contributes to the inhibitory activity of said inhibitor.

In a more preferred embodiment, a molecule which is capable of forming an interactive site
15 with a phospholipase enzyme mimics the 3-dimensional structure (i.e. tertiary structure) of NSI, NAI, OSI, OMI and/or PTI and, as a consequence, is capable of reproducing PLA₂ inhibitor:PLA₂ inhibitory interaction.

In this regard, whilst not being bound by any theory or mode of action, the mechanism of
20 interaction between the PLA₂ inhibitor and the PLA₂ enzyme at least appears to be unique compared to the mode of interaction of other PLA₂ inhibitors with specific enzymes which they inhibit, thereby accounting for the generality of the PLA₂ inhibitory activity of the present invention. Those skilled in the art will be aware that once the structure of the interactive site between a PLA₂ inhibitor and a PLA₂ enzyme is established by standard X-ray
25 crystallographic procedures, it is possible to synthesize peptides or other molecules (mimotypes or mimetics) which are capable of reproducing the inhibitory function of the inhibitors. Such mimotypes, whilst capable of forming a complex with interactive site with a phospholipase enzyme may not comprise the same amino acid sequence (i.e. primary structure) as the inhibitor α -chain and/or β -chain polypeptide(s), particularly in light of the
30 finding by the inventors that both α -chain and β -chain polypeptides of the subject PLA₂ inhibitors are required for full inhibitory activity against the PLA₂ enzymes. Furthermore,

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those skilled in the art will be aware that mimotypes may also comprise synthetic molecules such as chemical compounds or anti-idiotypic antibodies of the phospholipase inhibitor of the present invention capable of forming an interactive site with a phospholipase. Those skilled in the art will also be aware that mimotypes may be presented on a carrier molecule or
5 embedded therein, such that the mimotype moiety is presented in a functional conformation capable of inhibiting the phospholipase activity. Accordingly, the present invention clearly extends to any molecule or composition of matter which at least comprises a mimotype or mimetic of NSI, NAI, OSI, OMI and/or PTI or the interactive site thereof.

- 10 Carrier molecules for presenting a mimotype may comprise the amino acid sequences presented as an in-frame fusion polypeptide with a polypeptide mimotype or alternatively, associated with a polypeptide mimotype by means of a disulfide bridge or other covalent bond formation, van der Waals interaction or ionic interaction, amongst others. Alternatively, wherein the mimotype moiety is a chemical compound, the mimotype may be
15 embedded into a polypeptide carrier by any means known to those skilled in art.

Carrier molecules for presenting a mimotype may also comprise polysaccharide molecules, nucleic acid molecules such as RNA or DNA, biologically inert carriers such as tungsten or gold, amongst others, polymers such as starches, dextrans, glycogen, Percoll (Trademark of
20 Pharmacia Fine Chemicals) or Ficoll (Trademark of Pharmacia Fine Chemicals), amongst others, agarose, polyacrylamide or carriers known to those in the pharmaceutical and/or biomolecular engineering industries.

In a particularly preferred embodiment, the present invention provides a PLA_2 inhibitor
25 having a β -chain comprising an amino acid sequence substantially as set forth in one of SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 or 87 or an amino acid sequence having at least 40% similarity to one or more of the above listed sequences.

In a related embodiment, the present invention contemplates a PLA_2 inhibitor having a β -
30 chain encoded by a nucleotide sequence comprising a sequence as set forth in one of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence having

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at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to one or more of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90.

- 5 Another aspect of the present invention is directed to an isolated PLA₂ inhibitor comprising an α -chain comprising an amino acid sequence set forth in one of SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 or 85 or an amino acid sequence having at least about 40% similarity to one or more of the above listed sequences.
- 10 In a related aspect of the present invention, there is provided an isolated PLA₂ inhibitor having an α -chain encoded by a nucleotide sequence comprising as set forth in one of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing to one or more of SEQ ID NOs. 5-7, 13-15, 39-
15 41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 under low stringency conditions at 42°C.

Preferably, the PLA₂ inhibitors according to these aspects of the present invention are capable of inhibiting more than one type of PLA₂ enzyme and in particular two or more of PLA₂ Type I, II and/or III enzymes.

20

- The term "similarity" as used herein includes exact identity between compared sequences at nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or
25 conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each or at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred
30 programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with largest

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number of matched bases and fewest gaps. Gap uses the alignment method of Needleman and Wunsch (1970). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website <http://mell.angis.org.au..>

5

The present invention further extends to hybrid PLA₂ inhibitors.

Accordingly, another aspect of the present invention is directed to a PLA₂ inhibitor comprising an α - and β -chain wherein the α -chain comprises an amino acid sequence selected
10 from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of the above sequence and a β -chain comprising an amino acid sequence selected from SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least 40% similarity to one or more of the latter sequences.

15

In one embodiment, the α - and β -chain are as occurring in the native PLA₂ inhibitor. In another embodiment, the α -chain of one PLA₂ inhibitor is associated with a β -chain of another PLA₂ inhibitor.

20 In a particularly preferred embodiment, the present invention provides a PLA₂ inhibitor comprising α - and/or β -chains having amino acid sequences or encoded by nucleotide sequence substantially as set forth for NSI, NAI, OSI, OMI and PTI in Table 2.

Preferred percentage similarities include at least about 50%, at least about 60%, at least
25 about 70%, at least about 80%, at least about 90% or above such as 92%, 93%, 94% or 95-100%.

The present invention clearly extends to the full-length amino acid sequences of both the precursor and mature α -chains and β -chains of NSI, NAI, OSI, OMI and PTI and to
30 heteropolymers and recombinant and isolated forms thereof, including fusion molecules.

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In the present context, "homologues" of a phospholipase inhibitor or a PLA₂ inhibitor include those molecules which have a similar inhibitory activity to one or more of NSI, NAI, OSI, OMI and PTI, such as molecules having at least about 40% similarity to NSI, NAI, OSI, OMI or PTI at the amino acid or nucleotide level. Homologues may comprise fusion
5 polypeptides between α -chains and β -chains with or without additional "spacer" sequences therebetween to facilitate folding and the ability of the fusion polypeptide to form an interactive site with a phospholipase enzyme. A homologue may be isolated or derived from the same species as the particular PLA₂ inhibitor or from a different species.

10 Furthermore, the amino acids of a homologous polypeptide may be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, charge or antigenicity, and so on.

"Analogues" encompass PLA₂ inhibitors which are at least about 40% similar to one or
15 more of NSI, NAI, OSI, OMI and/or PTI or their interactive sites, notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein. "Analogues" also encompass polypeptide mimetics or mimotypes of the phospholipase inhibitor herein described.

20 The term "derivative" in relation to a PLA₂ inhibitor shall be taken to refer herein to mutants, parts or fragments derived from a functional PLA₂ inhibitor or homologues or derivatives thereof which may or may not possess PLA₂ inhibitory activity. Derivatives include modified molecules in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as
25 radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject PLA₂ are particularly contemplated by the present invention. Additionally, derivatives of a PLA₂ inhibitor which comprise fragments or parts of an amino acid sequence disclosed herein are within the scope of the present invention, as are homopolymers and heteropolymers comprising two or more copies of the subject
30 polypeptides. Procedures for derivatizing peptides, polypeptides and proteins are well-known in art.

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Particularly preferred analogues and derivatives of the PLA₂ inhibitors exemplified herein comprise an amino acid sequence which is capable of binding to the active site of a phospholipase enzyme and/or capable of forming an interactive site with a phospholipase enzyme.

5

Substitutions which may be included in a homologue, analogue or derivative of a subject PLA₂ inhibitor encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a phospholipase inhibitory protein is replaced with another naturally-occurring amino acid of similar character, for example, the substitutions Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln and Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a phospholipase inhibitor is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group (eg. a substitution of a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

20

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Naturally-occurring amino acids include those listed in Table 3. Non-conventional amino acids encompassed by invention include, but are not limited to those listed in Table 4.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to N-terminus, C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino-or carboxyl-terminal fusions and of the order of 1-4 amino acid residues.

30

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The phospholipase inhibitor protein of the present invention or a homologue thereof may comprise polypeptide chains having an estimated molecular weight of from about 10 kDa to about 50 kDa as determined by SDS/PAGE or by mass spectrometry.

5

When the phospholipase inhibitor is in multimeric form, such as a heteropolymer of α -chain and β -chain polypeptides, it is also preferred that it exist as a trimeric protein having a molecular weight in the range of from about 30 kDa to about 150 kDa, more preferably about 45 kDa to about 90 kDa.

10

In a particularly preferred embodiment of the present invention, the phospholipase inhibitor or a homologue or analogue thereof is a heterotrimeric $\alpha_2\beta$ protein.

Accordingly, another aspect of the present invention contemplates a PLA_2 inhibitor or a recombina_{nt}, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof comprising structure:



20 wherein

α is an α -chain of a PLA_2 inhibitor;

β is a β -chain of a PLA_2 inhibitor;

m is an integer from 0 to 10;

n is an integer from 0 to 10

25 with proviso that if m and n are not 0, then $m > n$ and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 30 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences. Preferably, m is 2-4 and n is 1-2. More

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preferably, m is 2 and n is 1.

In one embodiment, α and β are from the same PLA₂ inhibitor.

- 5 In another embodiment, α and β are from different PLA₂ enzymes. Examples of different PLA₂ enzymes include PLA₂ Type I, II and III.

The the present invention clearly extends to fusion polypeptides comprising one or more α -chain and β -chain polypeptides and mimotypes thereof.

TABLE 3

5	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
10	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
15	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
20	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
25	Tyrosine	Tyr	Y
	Valine	Val	V
	Any amino acid as above	Xaa	X

TABLE 4

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5			
α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
10 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbomyl-	Norb	L-N-methylglutamine	Nmgln
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

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	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl) glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
5	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl) glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
10	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
15	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
20	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylassparagine	Masn
	L- α -methylasspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
25	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomo phenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
30	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys

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L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
5 L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomo	
		phenylalanine	Nmhph
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl)glycine	Nnbhm	carbamylmethyl)glycine	Nnbhe
10 1-carboxy-1-(2,2-diphenyl-			
ethylamino)cyclopropane	Nmbc		

As exemplified herein, the NSI of the present invention is extremely stable to pH in the range
 15 4-12 and to high temperature, de-glycosylation or the action of denaturing agents. Similar
 properties exist for NAI, OSI, OMI and PTI.

The phospholipase inhibitor or its homologue, analogue, mimetic, derivative or chemical
 equivalent thereof as described herein is useful in a wide range of prophylactic and
 20 therapeutic applications, by virtue of the ability of the subject phospholipase inhibitor to at
 least inhibit Type I, II and/or III PLA₂ enzymes. The advantageous effects of the instant
 invention are achieved by the administration of isolated and/or recombinant phospholipase
 inhibitors or functional homologues, analogues, mimetics, derivatives or chemical equivalents
 thereof to a human or animal subject, either directly or *via* their genetic sequences. Where
 25 administration is direct, the phospholipase inhibitor may be administered alone or as a fusion
 molecule or in another format such as by means known to those skilled in art.
 Administration may be as a composition comprising at least the isolated or recombinant PLA₂
 inhibitor in combination with one or more pharmaceutically acceptable carriers and/or
 diluents. Alternatively, where it is desirable for the subject phospholipase inhibitor to be
 30 administered by genetic means, such means include *via* an attenuated virus, a recombinant
 viral vector, a nucleic acid molecule, a genetic construct or by bacterial vector.

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Administration means include injection, infusion, oral ingestion, suppository and nasal spray or drops amongst other routes.

Accordingly, a further aspect of the present invention contemplates a composition comprising
5 an isolated or recombinant phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

Alternatively, or in addition, the composition may comprise a mimotype of the phospholipase
10 inhibitor.

Preferably, the composition is injected or orally administered. Where the composition comprises genetic material such as DNA or RNA, preferably it is administered as part of a viral vector, live viral vector, live bacterial vector or naked or protected nucleic acid
15 molecule.

Conditions for which treatment might be required include any inflammatory condition, autoimmune condition, organ dysfunction or toxic poisoning which involves the action of a PLA_2 enzyme and in particular a Type I, II or III PLA_2 enzyme. More preferably, the
20 PLA_2 inhibitor of the present invention and compositions comprising same are useful for the treatment of rheumatoid arthritis, osteoarthritis, asthma, allergy, psoriasis, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome and neutralisation of allergic reactions to animals such as arachnids (eg. spiders, scorpions, mites, etc) insects (eg. wasps, bees, ants, fleas, etc), reptiles (eg. snakes, lizards, etc),
25 amphibians (eg. toads, frogs) or aquatic animals (eg. fish, cephalopods, box jellyfish, Portuguese man-of-war jellyfish, blue-ringed octopus, etc), amongst others, or the toxic effects of toxins produced by such animals.

In an even more preferred embodiment, the PLA_2 inhibitor of the present invention and
30 pharmaceutical compositions comprising same are useful for the treatment of inflammation associated with rheumatoid arthritis or osteoarthritis, for the treatment of snake bite,

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arthropod bite, insect stings and neutralisation of the toxic effects of notexin.

By "snake bite" is meant the allergic reaction or toxic effects of any snake bite, including the allergic reaction or toxic effects of a venom produced by a snake. The present invention is particularly useful for the treatment of the toxic effects of a wide range of snake venoms, including those produced by snakes from any one or more of the families Colubridae (colubrid snakes such as species of the genera *Heterodon*, *Natrix*, *Regina*, *Clonophis*, *Thamnophis*, *Lampropeltis*, *Opheopdris*, *Coluber*, *Masticophis*, *Drymobius*, *Salvadora*, *Phyllorhynchus*, *Elaphe*, *Hydrodunastes*, *Ptyas*, *Calamaria*, *Lycodon*, *Mehelya*, *Boaedon*, *Farancia*, *Fordonia*, *Erpeton*, amongst others), Elapidae (cobras such as species of the genera *Ophiophagus*, *Naja*, *Oxyuranus*, *Pseudohaje*, *Walterinnesia*, *Aspidelaps*, *Boulengerina*, *Dendroaspis*, *Bungaris*, *Calliophis*, *Maticora*, *Micurus*, *Micruroides*, *Acanthophis*, *Notechis* and *Australaps*, amongst others), Hydrophiidae (sea snakes such as species of genera *Laticauda*, *Aipysurus*, *Hydrophis* and *Enhydrina*, amongst others), Viperidae (vipers, such as species of the genera *Vipera*, *Echis*, *Cerastes*, *Bitis*, *Atractaspis* and *Causus*, amongst others) and Crotalidae (pit vipers such as species of genera *Crotalis*, *Sistrurus*, *Bothrops*, *Trimeresurus*, *Lachesus* and *Agkistrodon*, amongst others).

Even more preferably, the PLA₂ inhibitor of the present invention or its homologue, analogue, mimetic, derivative or chemical equivalent thereof and compositions comprising same are useful in treatment of snakes bite, wherein the snakes are from the family Viperidae, such as *Vipera* spp. and *Bitis* spp., in particular, *V. russelli*, *A. bilineatus* and *B. alternatus*; family Crotalidae, such as moccasin snakes and vipers (*Agkistrodon* spp.) and rattlesnakes (*Crotalus* spp.), in particular *Crotalus atrox*; or the family Elapidae, such as but not limited to King cobra (*Ophiophagus hannah*); True cobras (*Naja* spp); Asian or Indian cobra (*N. naja*); Egyptian cobra (*N. haje*); Spitting cobra (*N. nigricolli*); Black-lipped cobra (*N. malenoleuca*); Cape cobra (*N. nivea*); Gold's tree cobra (*Pseudohaje goldii*); Desert black snakes (*Walterinnesia* spp); Shield-nose snakes (*Aspidelaps* spp); Water cobras or water snakes (*Boulengerina* spp); Black mamba (*Dendroaspis polylepis*); Mamba (*D. angusticeps*); Kraits snake (*Bungarus* spp); Oriental coral snakes (*Calliophis* spp); Long-glanded coral snakes (*Maticora* spp); American coral snakes (*Micurus* spp); Southern coral snake (*M.*

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frontalis); Eastern coral snake or Harlequin snake (*M. fulvius*); Western coral snake (*Micruroides* spp); Arizona coral snake (*M. euryxanthus*); Death adder (*Acanthophis antarcticus*); Australian tiger snakes (*Notechis* spp); and Australian copperhead (*Australaps* spp), amongst others.

5

By "arthropod bite" is meant an allergic reaction or toxic effects of any arthropod bite, including the allergic reaction or toxic effects of a venom produced by said arthropod. The arthropod may be a spider, in particular a venomous spider such as a funnel web spider, red-back spider, amongst others or a scorpion, pseudoscorpion, mite, wasp, bee or ant, amongst

10 others.

By "insect sting" is meant an allergic reaction or toxic effects of any insect sting, including the allergic reaction or toxic effects of a venom produced by said insect. The insect may be a wasp, bee or ant, amongst others.

15

The pharmaceutical compositions of the present invention may also contain other active molecules or ingredients such as antibiotics to prevent infection at a wound site induced by the animal causing the sting or bite (e.g. the arachnid, insect, reptile, amphibian or aquatic animal) and/or antigen molecules, to promote protective immunity against one or more

20 antigens present in the venom produced by said animal.

The active ingredient(s) of composition is/are contemplated to exhibit excellent PLA_2 inhibitory activity in animals and humans when administered. Variations in dosage administration occur depending, for example, on activity of the phospholipase enzyme

25 required to be inhibited and I_{50} inhibitor, intended purpose of administration, such as whether for use as an anti-inflammatory agent or as an anti-toxin and particularly in the case of toxic poisoning and the delay between the onset of symptoms and the commencement of treatment. Dosage regimen may be adjusted without undue experimentation by those skilled in the art to provide the optimum therapeutic response. For example, several divided doses
30 may be administered in one or more of hourly, daily, weekly or monthly or in other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of

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situation.

For toxicological applications, those skilled in art will be aware that the optimum dosage required should be calculated based upon LD_{50} value of the particular toxin being neutralised
5 and such calculations are well-within the capacity of such persons. For example, successful neutralisation of notexin is observed in mice administered with between 1-fold and 4-fold the toxic dose of notexin.

The compositions may conveniently be presented in unit dosage form and may be prepared
10 by any methods well known in the art. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

15 Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of active ingredient; a powder or granules; a solution or a suspension in an aqueous or non-aqueous liquid. The active ingredient may also be presented as a bolus, electuary or paste.

20 A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent), preservative disintegrant (e.g. sodium starch glycolate, cross-linked
25 polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of powdered compound moistened with an inert liquid diluent.

Tablets or powders or granules may optionally be coated or scored and may be formulated so
30 as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

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Additionally, sweeteners or dietary formulae may be included to improve their palatability to a specific animal subject. Optionally, such solid compositions are provided with an enteric coating, to provide release in parts of gut or than stomach.

- 5 The active compounds may also be administered in dispersions prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.
- 10 Pharmaceutical forms suitable for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
- 15 microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by use of
- 20 surfactants. The prevention of action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by their use in compositions of agents delaying absorption, for example.
- 25 Sterile injectable solutions are prepared by incorporating active compounds in the required amount in appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilisation. Generally, dispersions are prepared by incorporating various sterilised active ingredient(s) into a sterile vehicle which contains the basic dispersion
- 30 medium and required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of

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preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

- 5 The compositions may also be delivered by a live delivery system such as using a bacterial expression system to express the PLA₂ inhibitor in bacteria which can be incorporated into gut flora. Alternatively, a viral expression system can be employed. In this regard, one form of viral expression is administration of a live vector generally by spray, feed or water where an infecting effective amount of live vector (e.g. virus or bacterium) is provided to the
- 10 animal. Another form of viral expression system is a non-replicating virus vector which is capable of infecting a cell but not replicating therein. The non-replicating viral vector provides a means of introducing to the human or animal subject genetic material for transient expression therein to produce the PLA₂ inhibitory protein. The mode of administering such a vector is the same as a live viral vector.

15

The carriers, excipients and/or diluents utilised in the compositions of the present invention should be acceptable for human or veterinary applications. Such carriers, excipients and/or diluents are well-known to those skilled in the art. Suitable carriers and/or diluents include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and

- 20 antifungal agents, isotonic and absorption delaying agents, and the like. Except insofar as any conventional medium or agent is incompatible with the active ingredient, use thereof in the composition is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

- 25 The compositions of this invention may include other agents conventional in the art. For example, compositions suitable for oral administration may include such further agents as dietary formulae, binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents
- 30 include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry,

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orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or its esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

A further aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a phospholipase inhibitor or a homologue or derivative of said phospholipase inhibitor.

The present invention extends to derivatives, homologues and analogues of the subject nucleic acid molecule.

The origin of the isolated nucleic acid molecule is not essential to the performance of the present invention, the only requirement being that the expression product of the subject nucleic acid molecule is at least capable of inhibiting a phospholipase enzyme such as PLA₂, in particular a Type I, II or III PLA₂ enzyme, and more particularly, two or more of Type I, II and/or III PLA₂ enzymes.

The person of ordinary skill in the relevant art would, without any undue experimentation, be capable of determining an appropriate source of the subject nucleic acid molecule and obtaining same in order to perform the embodiments described herein.

Preferably, the nucleic acid molecule is originally derived from a species of animal which produces a toxin which exerts its effect *via* activity of a PLA₂ enzyme. In a particularly preferred embodiment, nucleic acid molecule of the present invention is derived from a venomous animal such as a snake. In a particularly preferred embodiment, the isolated nucleic acid molecule is derived from *Notechis* spp. such as, but not limited to *N. scutatus* or *N. ater* or an *Oxyuranus* spp. Such as but not limited to *O. scutellatus* or *O. microlepidotus* or a *Pseudonaja* species such as but not limited to *P. textilis*. Ultimately, nucleic acid

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molecule may be derived or maintained in an animal cell line, insect cells, eukaryotic cells or bacterial cells.

This aspect of the present invention clearly extends to any isolated gene which encodes a
5 PLA₂ inhibitor or a peptide or polypeptide subunit thereof or a homologue or derivative of
said inhibitor including a fusion or hybrid molecule encoding a fusion or hybrid PLA₂
inhibitor.

Reference herein to a "gene" is to be taken in its broadest context and includes:

- 10 (i) a classical genomic gene consisting of transcriptional and/or translational
regulatory sequences and/or a coding region and/or non-translated sequences (i.e.
introns, 5'- and 3'- untranslated sequences);
- (ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) optionally
comprising 5'- or 3'-untranslated sequences of the gene; or
- 15 (iii) an amplified DNA fragment or other recombinant nucleic acid molecule
produced *in vitro* and comprising all or a part of the coding region and/or 5'- or 3'-
untranslated sequences of the gene.

The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of
20 a functional product. A functional product is one which comprises a sequence of nucleotides
or is complementary to a sequence of nucleotides which encodes a functional PLA₂ inhibitor
and in particular NSI, NAI, OSI, OMI and/or PTI or a homologue or derivative thereof.

Genes of the present invention may be derived from a naturally-occurring PLA₂ inhibitor-
25 encoding gene by standard recombinant techniques. Generally, a PLA₂ inhibitor-encoding
gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions,
deletions and/or additions. Nucleotide insertional derivatives of the PLA₂ inhibitor encoding
gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence
insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those
30 in which one or more nucleotides are introduced into a predetermined site in the nucleotide
sequence although random insertion is also possible with suitable screening of the resulting

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product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substitutions are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity or hydrophobicity.

Accordingly, the present invention extends to homologues, analogues and derivatives of a gene which encodes a phospholipase inhibitor as herein described.

10

For the present purpose, "homologues" of a gene as hereinbefore defined or of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a gene as hereinbefore defined or of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a gene as hereinbefore defined or of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues.

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Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are characterised by the removal of one or more
5 nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

Particularly preferred derivatives are those which encode polypeptides capable of forming an
10 interactive site with a phospholipase enzyme, for example by reproducing or imitating the NSI:PLA₂ interactive site or alternatively, derivatives which encode polypeptides capable of at least binding to the active site of a phospholipase enzyme.

In a further alternative embodiment, the present invention provides an isolated nucleic acid
15 molecule having a sequence of nucleotides or complementary sequence of nucleotides comprising one or more of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82 to 84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to any one or more of said sequences under low stringency conditions at 42°C.

20

Preferred sequence similarities or identifies include at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or above such as at least about 93%, 95% or 97-100%.

25 The present invention clearly extends to any partial or full-length cDNA and genomic clone equivalents of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82-84 or 88-90 and any homologues or derivatives thereof, in particular those homologues, analogues or derivatives which are obtainable using the genetic sequences herein provided.

30 In an alternative embodiment, the present invention extends to any isolated nucleic acid molecule which is at least capable of encoding an amino acid sequence set forth in any one of

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SEQ ID NOS: 1-4, 9-12, 17-38, 43-46, 51-53, 57-59, 63-66, 71-74, 79-81 or 85-87 which is at least capable of encoding the α -chain and/or β -chain polypeptide subunits of a phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof.

5

The present invention further encompasses any isolated nucleic acid molecules which comprise at least a part of a PLA₂ inhibitor-encoding gene sequence as herein described. Preferably, such parts comprise at least about 10 contiguous nucleotides in length, more preferably at least about 15 contiguous nucleotides in length, even more preferably at least about 30 contiguous nucleotides in length and still even more preferably at least about 50 contiguous nucleotides in length, of SEQ ID NOS: 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82-84 or 88-90 or of a sequence capable of hybridizing to these sequences under low stringency conditions at 42°C.

15 In a particularly preferred embodiment, the present invention provides a nucleic acid molecule which encodes an α -chain polypeptide of a PLA₂ inhibitor protein or a homologue or derivative thereof which nucleotide sequence has at least about 75% similarity to one or more of the nucleotide sequences set forth in SEQ ID NOS. 5-7, 13-15, 39-41, 47-49, 54-55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence capable of hybridizing under low
20 stringency conditions at 42°C to one or more of said sequence.

More preferably, percentage similarity is at least about 90%, including at least 91% or 93% or 95% or 100%.

25 In an alternative embodiment, the present invention contemplates an isolated nucleic acid molecule which encodes a β -chain polypeptide or a PLA₂ inhibitor protein or a homologue or derivative thereof which has at least about 75% similarity to any one of the nucleotide sequences set forth in SEQ ID NOS: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence capable of hybridising under at least low stringency conditions at 42°C
30 to one of said sequences.

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In a preferred embodiment of invention, the hybridisation stringency is at least medium stringency. More preferably, the hybridisation stringency is at least a high stringency.

In a particularly preferred embodiment, the nucleic acid molecule of the present invention is further characterised as a PLA₂ inhibitor encoding gene.

Yet another aspect of the present invention provides an nucleic acid molecule encoding hybrid PLA₂ inhibitors.

10 Accordingly, the present invention provides a nucleic acid molecule encoding a PLA₂ inhibitor having the structure:



15 wherein

α is an α -chain of a PLA₂ inhibitor;

β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence

20 selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

25

In another embodiment, the present invention is directed to a nucleic acid molecule encoding a PLA₂ inhibitor having the structure:



30

wherein

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α is an α -chain of a PLA₂ inhibitor;
 β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;
n is an integer from 0 to 10 with proviso that if m and n are not 0, then $m > n$ and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by a nucleotide sequence
5 selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and
88 or a nucleotide sequence having at least about 40% similarity to one or more of said
sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences
under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected
from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide
10 sequence capable of hybridizing to one or more of said sequences under low stringency
conditions at 42°C.

Reference herein to a low stringency at 42°C includes and encompasses from at least about
1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M
15 salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions.
Alternative stringency conditions may be applied where necessary, such as medium
stringency, which includes and encompasses from at least about 16% v/v to at least about
30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for
hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or
20 high stringency, which includes and encompasses from at least about 31% v/v to at least
about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for
hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.
In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, 1962).
However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in number of
25 mismatch base pairs (Bonner and Laskey, 1974).

The functional genetic sequences of the present invention are useful for expression of the
PLA₂ inhibitor in cells. Non-functional genetic sequences which do not express a functional
PLA₂ inhibitor are at least useful as, for example, genetic probes, primer sequences, antisense
30 or sense molecules or in the generation of immunologically interactive recombinant
molecules.

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In a particularly preferred embodiment, the PLA₂ inhibitor-encoding genes and homologues or derivatives thereof are employed to identify and isolate similar genes from other sources. The present invention extends to all such applications.

- 5 Related PLA₂ inhibitor-encoding genes are isolated by contacting genomic DNA, or mRNA, or cDNA, or an amplified gene product or a part, fragment or a source thereof, with a hybridisation effective amount of a probe and then detecting the hybridisation. The related genetic sequence may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell or a plant cell. In addition, the related genetic sequence may be bound
10 to a support matrix or membrane comprising, for example, nylon, nitrocellulose, polyacrylamide or agarose, amongst others. The probe is generally labelled with a reporter molecule capable of giving an identifiable signal (e.g. a radioisotope such as ³²P or ³⁵S or a biotinylated molecule).
- 15 An alternative method contemplated by the present invention involves hybridising a nucleic acid primer molecule of at least 10 nucleotides in length derived from the nucleotide sequence herein described to a nucleic acid "template molecule", said template molecule herein defined as a related PLA₂ inhibitor-encoding gene or a functional part thereof, or its complementary sequence. Specific nucleic acid molecule copies of the template molecule are
20 amplified enzymatically in a polymerase chain reaction, a technique that is well known to those skilled in the art and which is described by McPherson *et al.* (1991).

Preferably, the nucleic acid primer molecule or the molecule effective in hybridisation is contained in an aqueous mixture of other nucleic acid primer molecules. More preferably,
25 the nucleic acid primer molecule is in a substantially pure form. The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacterial cell, bacteriophage particle, yeast cell, animal cell, or a plant cell. For production of recombinant protein in isolated cells, the nucleic acid molecule of the present invention is placed, in the sense orientation, in operable connection with a suitable promoter sequence and introduced into a
30 suitable expression system, for example a bacterial, yeast, baculovirus, plant, animal or other expression system.

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Accordingly, a further aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which corresponds to, or is complementary to a phospholipase inhibitor-encoding gene or a
5 homologue or derivative thereof.

According to this embodiment, the coding region of a phospholipase inhibitor encoding gene may be placed in operable connection with a promoter sequence such that a gene product is capable of being expressed under the control of said promoter sequence.

10

Optionally, said genetic construct further comprises a terminator sequence.

In present context, the term "in operable connection with" is used to indicate that expression of the isolated nucleotide sequence is under the control of the promoter sequence with which
15 it is connected.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences
20 to the 3'-end of a primary transcript. Terminators active in plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the genetic constructs of the present
25 invention include SV40 polyadenylation signal, amongst others.

Reference herein to a "promoter" is to be taken in its broadest context and includes transcriptional regulatory sequences of a classical genomic gene, including a TATA box which is required for accurate transcription initiation in eukaryotic cells, with or without a
30 CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers). For expression in prokaryotic cells, such as bacteria, the promoter

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should at least contain the -35 box and -10 box sequences.

A promoter is usually, but not necessarily, positioned upstream or 5', of the phospholipase inhibitor encoding gene, the expression of which it regulates. Furthermore, the regulatory
5 elements comprising a promoter are usually positioned within 2 kb of start site of transcription of gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or a derivative which confers, activates or enhances expression of an isolated
10 nucleic acid molecule, in a cell, such as a plant, animal, insect, fungal, yeast or bacterial cell. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression of a nucleic acid molecule which expression it regulates and/or to alter the spatial expression and/or temporal expression of same. For example, regulatory elements which confer copper inducibility may be placed adjacent to a
15 heterologous promoter sequence driving expression of a nucleic acid molecule, thereby conferring copper inducibility on the expression of said molecule.

Placing an isolated nucleic acid molecule under the regulatory control of a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence.
20 Promoters are generally positioned 5' (upstream) to genes that they control. In construction of heterologous promoter/structural gene combinations, it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art,
25 some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

30

Examples of promoters suitable for use in genetic constructs of the present invention include

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viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may regulate the expression of the nucleic acid molecule constitutively, differentially with respect to the tissue in which expression occurs or, with respect to developmental stage at which expression occurs, or
5 inducibly such as in response to external stimuli including physiological stresses, plant pathogens, metal ions, effector molecules, amongst others.

Preferably, the promoter is capable of regulating expression of a nucleic acid molecule in a yeast or bacterial cell.

10

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* promoter, *tac* promoter, SV40 early promoter and the like.

The genetic construct contemplated herein is introduced into a suitable expression system for
15 a time and under conditions sufficient for expression of the PLA₂ inhibitor gene to occur.

Accordingly, a further aspect of invention contemplates a recombinant phospholipase inhibitor, produced by expressing a nucleic acid molecule described herein in a suitable host cell. The present invention extends also to a synthetic peptide fragment of said recombinant
20 gene product.

It is to be understood that the recombinant and isolated PLA₂ inhibitors described herein include homopolymers and heteropolymers of an α -chain and/or β -chain of a PLA₂ inhibitor. The α - and β - chains may be produced independently as recombinant proteins and
25 reassociated into active molecules. They may occur *in vitro* or *in vivo*. For example, the two chains could be expressed as separate proteins by the same recombinant cell (bacteria, yeast, insect or mammalian) using several different genetic constructs. A construct could be produced where the expressed protein consists of α - and β - chains joined by a linker peptide in a manner analogous to the structure of single chain Fv recombinant antibody fragments.
30 The two such molecules would then self-associate to form the appropriate structure.

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With particular regard to recombinant polypeptides, those skilled in the art will be aware of methods which may be employed to produce such multimeric proteins, for example, by co-expression of polypeptide subunits on separate genetic constructs or the same genetic construct, in a suitable cellular host. Alternatively, α - and β -chain subunits of a PLA₂ inhibitor may be expressed as a fusion polypeptide.

The present invention clearly extends to the recombinant production of polypeptide mimotypes of NSI, NAI, OSI, OMI and PTI or their homologues.

- 10 Still further aspect of the present invention provides a method of isolating a PLA₂ inhibitor from snake blood, serum or other blood component, said method comprising steps of:
- (i) preparing a serum sample from clotted blot; and/or
 - (ii) subjecting blood serum to ion-exchange chromatography.
- 15 The ion exchange chromatography may be performed using an anion exchanger or cation exchanger.

The composition of buffers used for each of the steps of the subject method may be determined by the person skilled in the art, without undue experimentation, the only requirement of such buffer compositions being that they are suitable for the maintenance of activity of the PLA₂ inhibitor being purified under chromatographic procedure employed. The buffer compositions may additionally include at least one, preferably two and more preferably three protease inhibitors to prevent proteolysis of enzyme during the purification procedure, in particular a trypsin inhibitor.

25

Preferably, the purification is carried out as described in Example 1, for the purification of the *Notechis scutatus* PLA₂ inhibitor NSI. A simple procedure is also effective for NAI, OSI, OMI and PTI.

- 30 The present inventors compared amino acid and corresponding nucleotide sequences for NSI, NAI, OSI, OMI and PTI and determined the consensus sequences for the alpha and beta

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chains. The present invention extends, therefore, to isolated polypeptides and corresponding genetic sequences which are encompassed by these consensus sequences.

Accordingly, another aspect of the present invention provides an isolated polypeptide capable
 5 of inhibiting two or more of PLA₂ Type I, II and/or III wherein said polypeptide has an alpha chain comprising the following amino acid sequence:

Xaa Ser Cys Glu Xaa Cys Xaa Asn Xaa Gly Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Glu
 Cys Ala Ser Xaa Glu Asp Gln Cys Gly Thr Val Leu Xaa Glu Xaa Ser Xaa Ala Pro Ile Ser
 10 Xaa Arg Xaa Ile His Arg Xaa Cys Phe Ser Ser Ser Xaa Cys Lys Leu Glu Xaa Phe Asp Ile
 Asn Ile Gly His Asp Ser Xaa Xaa Arg Gly Arg Ile His Cys Cys Xaa Glu Xaa Xaa Cys Glu
 Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Xaa Gly
 Xaa Phe Ser Val Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Xaa Glu Thr Lys Cys Ile Xaa
 Xaa Ala Gly Phe Arg Xaa Glu Arg Xaa Xaa Xaa Asp Xaa Xaa Tyr Asn Ile Lys Gly Cys Thr
 15 Ser Ser Cys Pro Glu Leu Xaa Leu Xaa Asn Arg Thr His Xaa Xaa Xaa Xaa Asn Xaa Leu Ile
 Xaa Xaa Glu Cys Thr Xaa Ala Xaa Lys Xaa Xaa Pro Ser Glu.

Preferably, the isolated polypeptide is encoded by the following nucleotide sequence:

20 CNCTCATGTGAAANTTGTNCNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
 NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTTC
 NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
 GCAAACCTNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
 GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTCCTGGACTGC
 25 CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
 GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
 NNTTGCGGGATTTCAGAAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
 AGGTTGCACTTCTTCTGTCCAGAACTGANGTTGANNNATAG

30 or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

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Yet another aspect of the present invention provides an isolated nucleic acid molecule encoding an alpha chain of a PLA₂ inhibitor said nucleic acid molecule comprising the nucleotide sequence:

5 CNCTCATGTGAAANTTGTCTNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
 NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTCA
 NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
 GCAAACNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
 GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCTGGACTGC
 10 CCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
 GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
 NNTTGCGGGATTTCAGAANNGAAAGANNNTNNNNNAGACATNGNTTATAATATCAA
 AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

15 or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

Still a further aspect of the present invention is directed to an isolated polypeptide capable of inhibiting two or more of PLA₂ Type I, II and/or III wherein said polypeptide has a beta chain
 20 comprising the following amino acid sequence:

Leu Glu Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Xaa Asn Xaa Xaa Thr Lys Thr
 Cys Asp Ala Asn Gln Asp Xaa Cys Val Thr Xaa Gln Thr Glu Val Ile Arg Ala Pro Val Ser
 Leu Xaa Xaa Ile Ser Lys Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Xaa Glu Thr
 25 Ser Pro His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu Cys Lys
 Thr Leu Pro Pro Pro Val Leu Gly Xaa Lys Val Xaa Pro Pro Asn Gly Leu Gln Cys Pro Gly
 Cys Xaa Gly Leu Ser Ser Lys Glu Cys Thr Glu His Xaa Val Ser Cys Arg Gly Ser Glu Asn
 Gln Cys Leu Ser Xaa Ile Gly Lys Glu Phe Gly Xaa Phe Phe Arg Ala Leu Ser Tyr Lys Gly
 Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Xaa Phe Trp Asn Val Leu Glu Xaa Val
 30 Glu Val Asp Phe Lys Cys Xaa Pro Ala Leu Pro Lys Ser Ser Gln.

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Preferably, this isolated polypeptide is encoded by the following nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAAGTGAAGTGATA
5 AGAGCCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACCTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCACCCAACGGACTTCAGTGTCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAAGT
10 GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

15 or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

Even yet a further aspect of the present invention provides an isolated nucleic acid molecule encoding a beta chain of a PLA₂ inhibitor said nucleic acid comprising the nucleotide
20 sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAAGTGAAGTGATA
AGAGCCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
25 GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACCTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCACCCAACGGACTTCAGTGTCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
30 AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC

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CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

5

The present invention is further described with reference to the following non-limiting Examples.

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EXAMPLE 1**Purification of Phospholipase A₂ Inhibitor from Snake Blood**

Unless otherwise stated all experiments were performed at room temperature.

5

Tiger snake (*N. scutatus*) blood was collected and allowed to clot. The blood was then centrifuged at 1,500 x g for 15 minutes. The serum was then collected and stored at -20°C. Serum was extensively dialysed against 0.01M ammonium acetate (NH₄OAc), pH 7.0. The *N. scutatus* phospholipase A₂ inhibitor (NSI) was purified using anion exchange
10 chromatography.

Dialysed serum was loaded (up to 15mL at ~20mg/mL) onto a DEAE-Sephacel column (20 x 1.5cm) that has been equilibrated with 0.01M NH₄OAc, pH 7.0 at a flow rate of 0.5mL/min. A step gradient was then developed as follows: 0.1 NH₄OAc, 0.25M NH₄OAc,
15 0.5 NH₄OAc and 1.0M NH₄OAc (all pH 7.0). The eluent was monitored at 280nm with an Isco type 11 detector. The concentration of NH₄OAc was not increased until the preceding peak has fully eluted. NSI eluted the 0.5M NH₄OAc step. The procedure was performed at 4°C.

20 The sample was then concentrated by lyophilisation and then resuspended in water and stored at -20°C. Alternatively, if a large volume was collected (>15mL), the sample was concentrated using an Amicon ultrafiltration device fitted with a YM 10 membrane. This semi-purified preparation (SPP) of NSI was approximately 90-95% pure.

25 NSI can be purified to >98% purity using cation exchange chromatography. A Mono-S HR 5/5 column was equilibrated with 10mM sodium acetate pH 5.5. The SPP NSI fraction was applied and a gradient developed with 430mM sodium acetate pH 5.5 as follows:

- (i) 0-3 minutes 0%;
- (ii) 3-8 minutes 0-20%;
- 30 (iii) 8-20 minutes 20-40%;
- (iv) 20-25 minutes 40-60%; and

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(v) 25-30 minutes 60-100%.

NSI eluted in the 20-40% section of the gradient (Figure 1a and 1b).

EXAMPLE 2

5 Phospholipase A₂ assays and inhibition of Phospholipase A₂ activity by NSI

Phospholipase A₂ activity was assigned using a modification of the method of Radvanyi *et al.* (1989). This assay is based on the ability to measure the fluorescence emitted by an artificial substrate after it has been cleaved by a PLA₂ enzyme. The level of fluorescence is
10 proportional to the amount of cleaved substrate which is in turn proportional to enzymatic activity. The phospholipid substrate, labelled in the sn-2 position with 10-pyrenyldecanoic acid, forms micelles upon addition to the reaction medium. The fluorescence of the substrate is quenched by pyrene-pyrene interactions. Upon hydrolysis the free 10-pyrenyldecanoic acids are absorbed by bovine serum albumin (BSA) and the fluorescence emitted is measured.
15 The artificial substrate 1-hexadecanoyl-2-(1-predecanoyl)-sn-glycero-3-phosphocholine (10pPC [Molecular Probes, Inc.]) was dissolved (1mg) in 5.87mL 95% v/v ethanol to yield a 0.2M stock solution. Aliquots of 200μL were stored at -20 C for up to 3 months.

To 1mL of assay buffer (50mM Tris [hydroxymethyl]methylamine-HCl[Tris]), pH7.5,
20 100mM NaCl, and 1mM ethylenediaminetetra-acetic acid [EDTA]) the following were added sequentially; 16μL of a 1:0.6 (v/v) mixture of 10% (w/v) BSA and 1M CaCl₂ (0.1% w/v and
2μM final concentration, respectively), 10μL 10pPC stock solution, injected quickly to facilitate micellular formation. To this, 35μL of a test sample, PLA₂ source plus SPP or
25 water, or saline/BSA, was added. This solution was mixed well with shaking. The substrate was excited at 345nm and the fluorescent spectrometer for 4 minutes.

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EXAMPLE 3**Inhibition of a variety of snake venom phospholipase A₂ activities
by partially-purified *N. scutatus* phospholipase A₂ inhibitor**

- 5 Using the SPP fraction prepared according to Example 1, inhibition of the phospholipase A₂ activities of a wide range of snake venoms was tested. The venoms tested were; *N.scutatus* (homologous venom), *P.textilis*, *N.melanoleuca* (family; Elapidae), *V.russelli* (family; Viperidae), *A. bilineatus*, *B.alternatus* and *C.atrox* (family; Viperidae, subfamily; Crotalinae).
- 10 First, an appropriate dilution of venom was established for use in the assay described in Example 2. The criteria required a substantial change in fluorescent intensity over a relatively short period of time. Venoms were diluted to achieve a phospholipase A₂ enzyme activity sufficient to produce a change of 250 fluorescent intensity units over 70-80 seconds in the absence of any inhibitor. As such all venoms showed similar PLA₂ activity in the assay.
- 15 A 1mg/mL solution of each venom was made up fresh when it was to be tested. Dilutions (of the 1mg/mL solution) used in the assay are as follows; *N.scutatus* 1/200, *P.textilis* 1/20, *N.melanoleuca* 1/150, *V.russelli* 1/15, *A.bilineatus* 1/20, *B.alternatus* 1/10 and *C.atrox* 1/10.

The SPP fraction was also diluted prior to testing against each venom. The dilutions were;

20 1/2, 1/8, 1/12, 1/50, 1/100 and 1/200 of a 1.11mg/mL solution.

The SPP dilutions were incubated with each diluted venom sample in the ratio 2.5:1 (v/v) before assaying phospholipase A₂ enzyme activity. Three assays were performed for each dilution of SPP on each day. Control samples were assayed both before and after each

25 dilution was tested. The control consisted of venom plus water in the same ratio as the SPP:venom. Three batches were assayed daily with separate controls for each batch. All samples were prepared at the same time and then selected randomly for testing. All samples being tested were kept on ice. Samples not used immediately were stored at -20°C.

30 Results are expressed as percentage inhibition compared to control values. (Figure 2a and 2b). As shown in Figures 2a and 2b, the SPP fraction of *N.scutatus* phospholipase A₂

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inhibitor was most effective at inhibiting the activities of *N.scutatus* snake venom phospholipase A₂, with at least 80% inhibition of the related *N.melanoleuca* phospholipase A₂ being observed at all dilutions of SPP tested. Significant inhibition of phospholipase A₂ activities derived from the more distantly related species were also
5 observed at high concentrations of the SPP fraction, wherein 50% inhibition of *V.russelli* phospholipase A₂ was observed at a 1/25 dilution of SPP and a 50% inhibition of the *A.bilineatus* and *B.alternatus* phospholipase A₂ activities was observed at about a 1/12 dilution of SPP and a 50% inhibition of the *P.textilis* and *C.atrox* phospholipase A₂ activities was observed at about a 1/2-1/8 dilution of SPP.

10

These data indicate that the *N.scutatus* venom phospholipase A₂ inhibitor is a broad-spectrum inhibitor of snake venom phospholipase A₂ enzymes.

EXAMPLE 4

15 **Inhibition of non-snake venom phospholipase A₂ enzymes by
 N.scutatus phospholipase A₂ inhibitor**

Phospholipase A₂ enzyme activity assays were performed as described in Example 2. The assay was performed as above except that 10pPG (1-hexadecanoyl-2-(1-predecanoyl)-sn-
20 glycerol-3-phosphoglycerol, ammonium salt) was used as the substrate, because most of the non-snake venom PLA₂'s are not active on 10pPC. Also, saline, rather than water was used for the negative control.

PLA₂ enzymes were diluted to achieve an enzyme activity sufficient to produce a change of
25 250 fluorescent units over 70-80 seconds in the enzyme assay, in the absence of inhibitor. Samples tested were; *N.scutatus* venom (positive control), bee venom phospholipase A₂ (*Apis mellifera*), porcine pancreatic phospholipase A₂ PLA₂ (*Sus scrofa*), and osteo-arthritis synovial fluid aspirates and rheumatoid arthritis-synovial fluid aspirates. Dilutions of phospholipase A₂-containing samples which were used were as follows; *N.scutatus* venom
30 1/30, bee venom phospholipase A₂ 1/400, porcine pancreatic phospholipase A₂ 1/3, all 1mg/ml. Osteo-arthritis, undiluted to 1/10 and rheumatoid-arthritis-synovial, 1/30, 25-

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36mg/mL total protein. It should be noted that not all of the OA or RA samples meet with the activity criteria of 250 fluorescent intensity units over 70-80 seconds, however, the activity was consistent and measurable.

- 5 Dilutions of the SPP varied according to the phospholipase A₂ tested. Two dilution groups were used for a 7.13mg/mL solution of the SPP: Group 1; 1/14, 1/50, 1/330 and 1/660. Phospholipase A₂ sources challenged with this group were *N.scutatus* venom, porcine pancreatic phospholipase A₂ and bee venom phospholipase A₂. Group 2; 1/2, 1/7, 1/14 and 1/50. Phospholipase A₂ sources challenged with this group were, all OA and RA samples.

10

As shown in Figure 3a, the SPP fraction of *N.scutatus* phospholipase A₂ inhibitor strongly inhibited bee venom phospholipase A₂ at all concentrates tested. A 50% inhibition of porcine pancreas phospholipase A₂ was observed at a 1/4 dilution of SPP.

- 15 As shown in Figure 3b, the SPP fraction of *N.scutatus* phospholipase A₂ inhibitor significantly inhibited the three osteoarthritis samples tested, with about 40-60% inhibition of enzyme activity being observed at a 1/2 dilution of SPP. In two of the three samples tested, about 50% inhibition of phospholipase A₂ activity was observed at the 1/7 dilution level of SPP. Weak, albeit detectable inhibition of phospholipase A₂ in the rheumatoid arthritis
20 sample tested was also detected at the 1/2-1/8 dilution of SPP.

These data indicate that the *N.scutatus* venom phospholipase A₂ inhibitor is a broad-spectrum inhibitor of non-snake venom-derived phospholipase A₂ activities.

25

EXAMPLE 5

Mixed micelle assay of recombinant human type II phospholipase A₂ and inhibition of enzyme activity using *N.scutatus* phospholipase A₂ inhibitor

- 30 An alternative assay of phospholipase A₂ activity was a mixed micelle phosphatidylethanolamine (PE/sodium deoxycholate (DOC) assay modified from a method of

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Seilhamer *et al.* (1989). This assay is particularly suited to quantifying recombinant human phospholipase A₂ activity as it utilises a PE/DOC substrate. The PE substrate was prepared by dissolving freshly desiccated [¹⁴C]PE (Amersham) in 2% w/v DOC, then diluting this to 0.22 μmoles PE and 0.04% w/v DOC per sample in assay buffer (50mM Tris-HCl, pH 8.5, 5 2mM CaCl₂, 150mM NaCl, 0.04% w/v DOC). The sample was prepared by mixing 10 μL of the test material with 10 μL 10mM Tris-HCl, pH 7.4 and incubating for 10 minutes at 37°C. The reaction was started by the addition of 25 μL pre-warmed substrate and terminated by the addition of 10 μL 100mM EDTA. The reaction mixture (30 μL) was spotted and dried onto silica TLC plates. The plates were chromatographed using chloroform:methanol:acetic acid 10 (90:10:1) as solvent. The dried plates were then exposed overnight with Kodak X-OMAT AR film. Radioactivity at the origin was counted and the percent hydrolysis by phospholipase A₂ determined.

As shown in Figure 4, the recombinant human phospholipase A₂ activities is significantly 15 inhibited at 0.1-1.0 μM concentrations of *N.scutatus* phospholipase A₂ inhibitor. The IC₅₀ of *N.scutatus* phospholipase A₂ inhibitor for recombinant human non-pancreatic phospholipase A₂ is approximately 1.5 μM.

EXAMPLE 6

20 pH Optimum and temperature stability of *N.scutatus* venom phospholipase A₂ inhibitor

The pH stability was investigated by altering the pH of the solution in which the SPP (0.4mg/mL) was dissolved and then testing this in the phospholipase A₂ assay. The assay was 25 performed as described in Example 2, using *N.scutatus* venom as the phospholipase A₂ source (1/200 dilution of a 1mg/mL with 10pPC as substrate). All samples were performed in triplicate with appropriate positive and negative controls. The pH values tested were: 2, 4, 6, 7, 8, 9, 10 and 12.

30 The temperature stability was assessed in the same manner as the pH stability. Samples were heated, or cooled, at the appropriate temperature and then immediately tested in the

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phospholipase A₂ assay. Temperatures examined were; 4°C, 25°C, 37°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C.

For both experiments samples were not preincubated with the venom as the stability of the phospholipase A₂ under the varying pH and temperature values could not be assured. However, the ratios phospholipase A₂ to inhibitor used in the preceding Examples were maintained in this procedure.

As shown in Figure 5a, NSI was stable in the pH range 4.0-12.0, with activity declining at extreme acidic pH values. Figure 5b shows the temperature stability of the inhibitor at all temperatures tested. Thus, NSI is a highly-stable protein.

EXAMPLE 7

Activity of the *N.scutatus* phospholipase A₂ inhibitor

following de-glycosylation of the α -chain

The α -chain was deglycosylated with N-glycosidase F (cleaves N-linked sugars) or O-glycosidase (cleaves O-linked sugars) as follows: 10 μ g (10 μ L) of the SPP was denatured with an equal volume of 1% (w/v) SDS followed by boiling for 2 minutes. To this 90 μ L 20mM sodium phosphate buffer, pH 7.2, 50mM EDTA, nonidet P-40, 0.5% v/v was added followed by a further 2 minutes boiling. The SPP was then incubated with 0.4U N-glycosidase or 2.5mU O-glycosidase for 16 hours at 37°C. A sample was then run on SDS-PAGE under reducing conditions. The gel was then blotted onto nitrocellulose and sugar residues detected with the Boehringer Mannheim DIG glycan detection kit as per manufacturers instructions. Appropriate controls were performed. A duplicate gel was run and silver stained to determine the shift in molecular weight of the α -chain following deglycosylation.

It was determined that only N-linked sugars were present on the α -chain. As such, the α -chain was deglycosylated with N-glycosidase F as outlined above except that SDS and nonidet P-40 were omitted as were the boiling steps. This was to ensure that NSI was not

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irreversibly denatured by boiling or SDS treatment. Deglycosylation was confirmed with the DIG glycan detection kit and the shift in molecular weight following SDS-PAGE. The sample was then assayed for inhibitory activity on *N.scutatus* venom (1/300 dilution of 1mg/mL solution dissolved in saline/0.1% w/v BSA) as described in Example 3. Native NSI
5 was used as the positive control.

The formation of the NSI intact complex following deglycosylation of the α -chain was determined using size exclusion chromatography. The deglycosylated SPP (containing NSI) was run on a Superdex 75 column (3.2mm x 30mm) using the Pharmacia SMART HPLC
10 system in 0.1M NH_4OAc pH 7.0. The column was calibrated with molecular weight standards. Native SPP was run as a positive control.

As shown in Figure 6a, de-glycosylated NSI retained activity compared to the native inhibitor, consistent with observations in respect of both *A.bilineatus* and bee venom
15 phospholipase A_2 inhibitors.

However, the de-glycosylated NSI exhibited a different elution profile from Superdex 75 compared to the native inhibitor, with significantly higher molecular weight species being present, possible due to the formation of functional high molecular weight aggregates
20 involving the de-glycosylated α -chain (Figure 6b). Additionally, the size of the assembled NSI complex differed slightly from native NSI (Figure 6b) due to the altered glycosylation status of the assembled complex.

EXAMPLE 8

25 **Determination of the *N.scutatus* phospholipase A_2 inhibitor complex formation with notexin**

The native molecular weight of NSI was determined using size exclusion chromatography using a Pharmacia Superose 12 HR10/30 column attached to a Waters 600 series HPLC
30 system. Elution buffer was 0.1M NH_4OAc , pH 7.0 at a flow rate of 0.5mL/min. NSI (60 μ g) was loaded on the column. The column was calibrated with molecular weight standards. The

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formulation of a stable complex between NSI and notexin was also investigated using size exclusion chromatography. The SPP (150 μ g) and notexin (100 μ g) were incubated for 30 minutes followed by elution on the Superose column. As shown in Figure 7, the NSI and notexin mixture eluted from Superose 12 immediately before NSI, confirming the ability of NSI to bind to notexin.

The peaks were collected and components identified by SDS-PAGE followed by silver staining to confirm their identities.

10

EXAMPLE 9

**Amino acid sequence determination of the α -chain of
N.scutatus phospholipase A₂ inhibitor**

The α -chain was prepared essentially as outlined in the following Example (Example 10) for the β -chain, except that the α -chain was subjected to cleavage by either trypsin or endoproteinase Glu-C. The endoproteinase Glu-C and trypsin digestion profiles of the α -chain are presented in Figures 8 and 9, respectively. The amino acid sequences of various isoforms of the NSI α -chain are shown in SEQ ID NOs:1-3 and 9-11 (see Table 2).

20

EXAMPLE 10

**Amino acid sequence determination of the β -chain of
N.scutatus phospholipase A₂ inhibitor**

The β -chain was purified from the SPP using RP-HPLC. A RP-300 7 μ m AQUAPORE (Brownlee Labs) column was connected to a Waters 600 series HPLC system. The buffers, 0.1% v/v trifluoroacetic acid (TFA) and 80% v/v acetonitrile, 0.08% v/v TFA were filtered before use. The column was equilibrated in 0.1% v/v TFA at a flow rate of 0.5 mL/min. SPP (200 μ L; 200 μ g) was loaded onto the column, after centrifugation at 10,000 x g for 10 minutes (Eppendorf Centrifuge 5415C), and eluted with a gradient of 80% v/v acetonitrile, 0.08% v/v TFA over 65 minutes. The gradient developed as follows; 0% for first 3 minutes, 0-40% over next 7 minutes, no change for 5 minutes, 40-80% over next 40 minutes 80-100%

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eluting buffer over the remaining 10 minutes. All gradients were linear.

The purified β -chain was then reduced and carboxymethylated. Reduction was performed by lyophilising the protein to near dryness followed by resuspension in 100 μ L 10mM DTT,

5 50mM Tris-HCl, pH 8.0 and then heated for 10 min at 60°C then 20 min at 37°C.

Alkylation was performed with the addition of 4-vinylpyridine to the above mixture to yield a final concentration of 1.5% v/v then incubate for 30 min at room temperature in the dark.

The sample was desalted by narrow bore RP-HPLC.

10 After desalting, the sample was lyophilised and resuspend in 50mM NH_4HCO_3 , pH 7.8 to which trypsin (Promega) was added at a 1:30 ratio (enzyme to protein). The protein was then incubated for 18 hours at 37°C. The digestion was stopped by the addition of TFA to a final concentration of 10% v/v. The peptides were then separated by RP-HPLC (C18, 2.1 x 100mm) on a Pharmacia SMART system. The resulting purified peptides were then N-
15 terminally sequenced. The reduced and alkylated β -chain was also subjected to cleavage with cyanogen bromide (CNBr). The desalted protein was lyophilised and resuspended in 70% v/v formic acid. To this 0.5 mg CNBr was added and incubated for 20 hours at room temperature. The resulting peptides were separated as described above and then N-terminally sequenced. (Figure 7). The amino acid sequence of the β -chain is shown in SEQ ID NO:4
20 and the leader sequence is shown in SEQ ID NO:12 (Table 2).

EXAMPLE 11

Isolation and characterisation of a cDNA clone encoding *N.scutatus* phospholipase A₂ inhibitor

25

Production of cDNA library

A cDNA library was produced from *N.scutatus* liver. The liver was collected and immediately placed in liquid nitrogen. A piece was taken and total RNA isolated using a

30 PolyA tract mRNA isolation system (Promega) as per manufacturers instructions. From this cDNA was produced using a cDNA synthesis System Plus kit (Amersham) as per

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instructions. The cDNA was then adapted with *EcoRI* adaptors and size selected using the Riboclone *EcoRI* adaptor ligation system I (Promega). The size selected cDNA was then used for the production of a cDNA library in the bacteriophage λ ZAPII (*EcoRI* digested and CIAP treated). The library was then titred and amplified as per instructions.

5

Screening of library, PCR and oligomers

Degenerate primers were designed from known N-terminal sequence of the α -chain and internal peptide data. Homology with the PLI from *Crotalus durissus terrificus* was used to aid in the primer design. The DNA sequence was known for the Crotalid PLI and as such minimally degenerate primers could be designed. cDNA prepared from *N.scutatus* liver was used as the template for the PCR. The forward primer sequence is 5'-CCAGAAGATCAG/ATGTGGC-3' [SEQ ID NO:91] and the reverse primer sequence is 5'-ATIGCGATGTCTCCAGG-3' [SEQ ID NO:92]. PCR was performed in a Hybaid Omni-
15 Gene PCR machine with the following cycle conditions [95°C x 2min] x 1, [61°C x 60sec, 72°C x 45sec, 95°C x 30sec] x 34, using tube temperature control and *Taq* polymerase (Qiagen). Product size and purity was assessed with TBE-PAGE. Ubiquitin primers were used as positive controls.

20 Using these primers, a specific 357bp product was amplified. The product was sequenced [SEQ ID NO: 5] using automated Li-cor sequencers. The sequence was confirmed to be the α -chain. As such, this product was used as a probe for the cDNA library. The product was labelled with a DIG labelling kit (Boehringer Mannheim) to enable detection of the probe. The library was then screened as per instructions. Three rounds of screening were
25 performed.

To establish the DNA sequence for the α -chain, 5' and 3' RACE was performed. The Marathon cDNA Amplification Kit (Clontech) was used as per manufacturers protocol. Degenerate primers, as used in Example 11, were used with the manufacturers supplied PCR
30 primer for either 5' or 3' RACE. For 5' RACE the PCR protocol was as follows: [95°C x 2.5 min] x 1, [94°C x 30s, 61°C x 45s, 72°C x 2 min] x 34. For 3' RACE the annealing

- 66 -

temperature (61°C) was increased to 65°C, all other parameters remained the same. After amplification of the partial gene it was sequenced using automated Li-cor DNA sequencers. The various isoforms of the α chain are shown in SEQ ID NOs: 5-7 and of the β chain in SEQ ID NO:8 (Table 2). Table 2 also summarizes the SEQ ID NOs for the leader sequences

5 from the α -chain and β -chain of NSI and its isoforms.

EXAMPLE 12

In vivo protection against notexin by NSI

10 The SPP was again tested against notexin to evaluate the effectiveness with which NSI was able to protect *in vivo*. Neonatal QS mice (3-5g) were used as the assay system, neonates are used in preference to adult mice as they are a more sensitive assay and also to conserve the limited amount of inhibitor available.

15 First, a toxic dose (TD) was established, by testing dilutions above and below the published LD₅₀ values for notexin [0.188mg/kg in saline, injected sub-cutaneously (S.C.); Sutherland, (1990)]. The dose was given in a total volume of 50 μ L injected s.c., the mice were incubated for 17 hours on a heating pad maintained at 30°C. The TD determined for notexin was 0.015 μ g/mouse. The TD was then used in the protection studies.

20

Protection studies were performed in triplicate with the following samples; 1x, 2x, 3x and 4x TD plus SPP (1mg/mL), positive control (TD plus saline), SPP control (SPP plus saline) and two negative controls (human serum and BSA, each at a concentration of 1mg/mL, plus TD). 160 μ L toxin was added to 160 μ L of the appropriate sample, or 160 μ L SPP plus 160 μ L

25 saline, and incubated at 37°C for 1 hour. A total volume of 100 μ L was then injected into each mouse. Mice were incubated as described above.

As shown in Table 5, the SPP fraction of *N.scutatus* phospholipase A₂ inhibitor successfully protected mice against up to at least 4xTD of notexin.

30

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TABLE 5

In vivo protection of mice against notexin using *N.scutatus* venom
phospholipase A₂ inhibitor

5	Sample Tested	Protection (alive/total)
	4TD + SPP	3/3
	3TD + SPP	3/3
	2TD + SPP	3/3
	TD + SPP	3/3
10	BSA + TD	0/3
	Human Serum + TD	0/3
	Venom Control (TD)	0/3
	SPP Control	3/3

15 Samples were as follows: 1x, 2x, 3x and 4xTD plus SPP (1mg/mL); BSA, BSA (1mg/mL) plus TD; human serum, human serum (1mg/mL) plus TD; venom control, TD plus saline; SPP control, SPP plus saline. Equal volumes of each were incubated at 37°C for 1 hour before injection. TD=0.015µg notexin s.c.

20

EXAMPLE 13**Crystallisation experiments**

Protein solutions of greater than 95% purity and between 5-50 mg/ml are surveyed for crystallisation parameters by the hanging drop vapour diffusion method. The method
25 involves centrifugation of the protein solution to remove particulates; the setting up of reservoirs containing different buffers, precipitants and additives in multiwell plates; pipetting 2µl of reservoir solution onto cover slips plus 2µl of protein solution to the drop; inverting the coverslip over the well; and incubating and observing for crystal formation.

30 Examples of precipitants include salts such as ammonium sulphate, ammonium formate and sodium citrate; different molecular weight polyethylene glycol [PEG 4000, 3000, 8000,

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20000]; organic solvents [MPD, ethanol] and mixtures of these.

Additives tested may include 0.25-1% v/v non-ionic detergent [eg. β -octyl glucoside]; dioxane; metal ions such as Ca^{2+} , Zn^{2+} ; reducing agents [dithiothreitol] and glycerol.

5

Other variables include pH, buffer type and temperature, amongst others.

As well as testing for crystal formation of purified NSI, co-crystallisation of NSI and PLA_2 is investigated to determine the structure of the interactive site. The two proteins are mixed
10 in equimolar quantities and the parameter survey procedure described above repeated.

Parameters which produce crystals are used with greater quantities of protein to produce large crystals suitable for X-ray diffraction experiments. These crystals are mounted, exposed to an X-ray source and diffraction data collected.

15

The data obtained are manipulated by phase determination, phase improvement extension, followed by interpretation of the electron density map. This process is aided by the amino acid sequence data and at 3Å resolution, an atomic model can be constructed. The atomic model of the co-crystal structure is based in part on the known solution of the human type II
20 PLA_2 structure. The atomic model can be refined by various procedures including energy minimisation.

EXAMPLE 14

25

Use of Crystal Structure Data

The crystal structures, particularly of the co-crystallised inhibitor and PLA_2 permits the determination of the structure of the interactive site. At the gross level, the molecular surfaces which make contact or are in close proximity are visualised, while at the finest
30 resolution of the structure, hydrophobic and ionic interactions between specific amino acid side chains are determined. This information specifies the critical residues of both the NSI

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and PLA₂ molecules which involved the protein-protein interaction resulting in PLA₂ inhibitory activity.

This atomic model is used to model further the possible interaction between human type II PLA₂ and the other snake PLA₂ inhibitors for which amino acid sequence data is obtained by the present inventors. These models of the interaction between the PLA₂ and the inhibitors are compared to their biological activity to gain a greater understanding of the inhibitory mechanism. Detailed information on the molecular interactions of these proteins, in particular elucidation of the structure of the interactive site, assists the rational design of synthetic proteins, peptides and organic molecules which utilise the inhibitory mechanism of NSI and other phospholipase inhibitory proteins of the invention.

EXAMPLE 15

Association (or Re-association) Experiments

Using native NSI, the α and β chains are separated and purified, then mixed under various conditions (e.g. guanidium chloride) to promote the re-association of the chains into the native tetrameric structure. The inhibitory activity of the re-associated molecule is tested. This information is used to produce novel active recombinant phospholipase inhibitors; based upon the α -chain and β -chain sequences of NSI.

EXAMPLE 16

Mixing Experiments

The conditions which permit reassociation of separated α and β chains are used in mixing experiments, using purified α - and β - chains from homologues of native phospholipase inhibitor polypeptides of the invention. For example, the α -chain from the tiger snake combined with the β -chain from either the Inland Taipan or Brown Snake. All permutations are produced and tested for inhibitory activity. Novel inhibitory proteins, having altered specificity or activity are thus obtained.

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Similar combinatorial mixing is performed with expressed recombinant α - and β - chains and with combinations of native and recombinant α - and β - chains, to identify phospholipase inhibitors having altered specificity and/or activity.

5

EXAMPLE 17**Cloning of PLA₂ inhibition from *Notechis ater* and from *Psuedonaja textilis***

Using similar procedures as described above, the PLA₂ inhibitor gene from *N. ater* (NAI) and *P. textilis* (PII) were cloned.

10

The nucleotide sequence of NAI α chain, isoforms i (NAI α i), ii (NAI α ii) and v (NAI α v) are shown in SEQ ID NOs:39-41, respectively. The nucleotide sequence of the leader sequence is shown in SEQ ID NO:47 (NAI α iL), 48 (NAI α iiL) and 49 (NAI α vL). The nucleotide sequence of the β -chain is shown in SEQ ID NO:42 (NAI β) and its leader sequence is shown 15 in SEQ ID NO:50 (NAI β L).

The corresponding amino acid sequences are shown in SEQ ID NOs: 17-38 and 43-46 (see Table 2).

20 The nucleotide and amino acid sequences of the PLA₂ inhibitor from *P. textilis* (PTI) are shown in SEQ ID NOs as summarized in Table 2.

EXAMPLE 18**Isolation of the gene encoding the alpha chain of PLA₂ inhibitor from coastal
25 and inland taipan snakes**

Total RNA was prepared from the liver of both Coastal Taipan and Inland Taipan snakes. Isolation of mRNA was carried out using standard techniques and purified mRNA was then transcribed into cDNA using the Reverse Transcriptase enzyme.

30

Primers for the isolation of the α -chain of the inhibitor protein were designed by using the

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DNA sequence previously determined for the α -chain of NSI (Tiger snake). A forward primer (a) was designed to include the start codon at the beginning of the secretion signal and a restriction site to allow directional cloning of the gene into the expression vector. The reverse primer (b) was complementary to the carboxy terminus of the protein and contained the translational stop codon:

(a) Forward primer (Tai for): 5'- CGCGGATCCATGAAATCCCTA-3' [SEQ ID NO:93]

(b) Reverse primer (Tai rev): 5'- CCGGAATTCTTATTATTCAGAAGG-3' [SEQ ID NO:94]

Amplification of the gene encoding the α -chain was carried out under standard conditions using the forward and reverse primers and the mRNA/cDNA hybrid as template. An amplified DNA product of approximately 610 bp was produced and isolated by agarose gel purification. The purified DNA was ligated into the sequencing vector pGEM-T and sequenced on an automated DNA sequencer.

15

The amino acid and nucleotide sequences of the coastal taipan (*O. scutellatus*) phospholipase inhibitor (OSI) are presented in SEQ ID NOs:51-53, 57-59, 54-56, 60-62 and 54-62. The amino acid and nucleotide sequences of the inland taipan (*O. microlepidotus*) phospholipase inhibitor (OMI) are presented in SEQ ID NOs:63-66, 71-74, 67-70 and 75-78. In both cases, the secretion signal consists of about 19 amino acids and this sequence is removed during processing of the protein.

The amino acid sequence alignment of the isoforms of NSI, NAI, OSI, OMI and PTI (α - and β - chains) is shown in Figure 11.

25

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

30

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (or than US): HSC (PLA) Pty Ltd AND Flair (PLA) R&D Pty Ltd AND Active (PLA) R&D Pty Ltd AND Heracles (PLA) R&D Pty Ltd AND Apelda (PLA) R&D Pty Ltd AND Edzell (PLA) R&D Pty Ltd AND Northmoor (PLA) R&D Pty Ltd

(US only): BROADY Kevin William, HAINS Peter Gregory

(ii) TITLE OF INVENTION: PHOSPHOLIPASE INHIBITOR

(iii) NUMBER OF SEQUENCES: 94

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: INTERNATIONAL/PCT
(B) FILING DATE: 27-NOV-1998

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PP0767
(B) FILING DATE: 5-DEC-1997

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Asp
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Ala Glu Asp Gln Cys Gly Thr Val Leu
 20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Lys
 35 40 45

Cys Phe Ser Ser Ser Ile Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
 50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
 85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
 100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
 115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile
 130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Asn Arg Thr His
145 150 155 160

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Glu Glu His Gly Asn Gly Leu Ile Lys Val Glu Cys Thr Glu Ala Ser
 165 170 175

Lys Ile Thr Pro Ser Glu
 180

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Glu
 1 5 10 15

Glu Ala Lys Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
 20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
 35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
 50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
 65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
 85 90 95

Gly Tyr His Cys Pro Gly Ile Phe Gly Val Phe Ser Val Asp Ser Ser
 100 105 110

Glu His Glu Ala Ile Cys Arg Gly Ser Glu Thr Lys Cys Ile Lys Ile
 115 120 125

Ala Gly Phe Arg Asn Glu Arg Phe Phe Gly Asp Met Gly Tyr Asn Ile

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130	135	140
Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Lys Leu Asn Arg Thr His		
145	150	155 160
Glu Glu His Gly Asn Gly Leu Ile Lys Val Glu Cys Thr Glu Ala Ser		
	165 170	175
Lys Ile Thr Pro Ser Glu		
	180	

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 183 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Ser Cys Glu Ile Cys His Asn Leu Gly Arg Asp Cys Glu Thr Glu
1 5 10 15
Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30
Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
35 40 45
Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60
Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80
Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95
Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

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Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
 115 120 125

Ala Gly Phe Arg Lys Glu Arg Phe Pro Gly Asp Ile Gly Tyr Asn Ile
 130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
 145 150 155 160

His Glu Glu Asp Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Asp Ala
 165 170 175

Ser Lys Ile Thr Pro Ser Glu
 180

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
 1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
 20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
 35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
 50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
 65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly His Lys Val Asn Pro Pro

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85	90	95
Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys		
100	105	110
Thr Glu His Leu Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser		
115	120	125
Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys		
130	135	140
Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp		
145	150	155
		160
Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu		
165	170	175
Pro Lys Ser Ser Gln		
180		

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCC AGAGTGACGA GACAGAGGAA	60
TGTGCCTCTG CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT	120
TCCTTCCGAT CCATTCATAG GAAGTGTTTC TCATCCAGCA TCTGCAAAC TGAACGCTTT	180
GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA	240
AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGCATTG TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360

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ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TATAGACATT 420
 GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAA TAGAACTCAC 480
 GAAGAACATG GAAATGGTCT 500

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACTCATGTG AAATTTGTCA CAATTTTGA AAAGACTGCC AGAGTGAGGA GGCAAAGGAA 60
 TGTGCGTCTC CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120
 TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAAC TGAACGCTTT 180
 GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240
 AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300
 CCTGGCATT T TGGTGTATT CTCACTGGAC AGTTCTGAAC ATGAAGCTAT TTGCAGAGGA 360
 AGTGAAACCA AATGCATTAA AATTGCGGGA TTCAGAAACG AAAGATTTTT TGGAGACATG 420
 GGTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAAGTTGAA TAGAACTCAC 480
 GAAGAACATG GAAATGGTCT 500

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACTCATGTG AAATTTGTCA CAATTTGGGA AGAGATTGTG AGACTGAGGA GGCAGAGGAA	60
TGTGCCTCTC CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT	120
TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACG CGAACGCTTT	180
GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA	240
AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGCATTTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATTTCG TGGAGACATC	420
GGTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT	480
CACGAAGAAG ATAGAAATGA	500

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTTGAGTGTG AGATTGTAT CGGGCTGGGC CTGGAATGTA ACACCTGGAC GAAAACCTGT	60
GATGCTAATC AAGATACTTG TGTACCTTT CAACTGAAG TGATAAGAGC CCCTGTGTCC	120
CTCTCTTTGA TCTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG	180
ACGAGTCCAC ATAATGAACT AACAGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA	240

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TGTAAACTC TGCCACCGCC TGTGCTTGA CACAAAGTCA ACCCACCCAA CGGACTTCAG 300
 TGTCTGGAT GCCTTGATT GTCCTCAAAA GAATGCACTG AACACCTGGT TTCCTGCCGG 360
 GGATCTGAAA ACCAGTGTTT GTCTATAATT GGAAGGAAT TTGGCCTTTT CTTAGAGCA 420
 TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTGAGAA GAGGTTCTGG 480
 AATGTTTTAG AGGATGTTGA 500

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
 1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg

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1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATGAAGTCCC TCTTATCTG TTGCCTCTTT GGCACCTTCT TAGCTACAGG CATGTGT

57

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His	Ser	Cys	Glu	Ile	Cys	His	Asn	Phe	Gly	Arg	Asp	Cys	Gln	Ser	Asp
1				5					10					15	

Glu	Ala	Glu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly
		20					25					

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Ser Cys Glu Ile Cys His Asn Leu Gly Lys Asp Cys Glu Thr Glu
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
20 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ile Thr Pro Ser Glu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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Arg Phe Asp Ile Asn Ile

1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ile Asn Leu Ala Gly Phe

1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Ser Lys Ile Thr Pro Ser Glu

1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Tyr Pro Gly Asp Ile Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val
20 25

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Leu Ser Tyr Lys
1 5

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(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Thr
1 5 10 15

Pro His Asn

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr Glu Val
1 5 10 15

Ile Arg

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Pro Val Thr Leu Gly Leu Ile
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Cys Thr Glu His Leu Val Ser Cys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Phe Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys
1 5 10

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(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly	Ser	Glu	Asn	Gln	Cys	Lys	Ser	Ile	Ile
1				5					10

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Val	Asn	Pro	Pro	Asn	Gly	Leu	Gln	Cys	Pro	Gly	Cys	Leu	Gly	Leu	Ser
1				5				10					15		

Ser	Leu	Glu	Cys	Thr	Glu
					20

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu Phe Gly Leu Phe Phe Arg
1 5

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Glu Gly Gly
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

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Met Glu Val Ser Thr Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
    35                40                45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
    50                55                60

Gly His Asp Ser Phe Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
    65                70                75                80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
                85                90                95

Gly Tyr His Cys Pro Gly Ile Leu Gly Leu Phe Ser Val Asp Ser Ser
                100                105                110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
                115                120                125

Ala Gly Phe Arg Arg Glu Arg Phe Pro Gly Asp Ile Ala Tyr Asn Ile
    130                135                140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
    145                150                155                160

His Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala
                165                170                175

Ser Lys Asn Thr Pro Ser Glu
                180

```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Asp

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1	5	10	15
Glu Thr Glu Glu Cys Ala Ser Ala Glu Asp Gln Cys Gly Thr Val Leu			
20	25	30	
Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Lys			
35	40	45	
Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile			
50	55	60	
Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala			
65	70	75	80
Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn			
85	90	95	
Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser			
100	105	110	
Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu			
115	120	125	
Ala Gly Phe Arg Lys Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile			
130	135	140	
Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Asn Arg Thr His			
145	150	155	160
Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala Ser			
165	170	175	
Lys Ile Thr Pro Ser Glu			
180			

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 183 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Glu Gly Gly
 1 5 10 15
 Val Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
 20 25 30
 Leu Glu Val Ser Thr Ala Pro Ile Ser Thr Arg Thr Ile His Arg Asn
 35 40 45
 Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
 50 55 60
 Gly His Asp Ser Tyr Met Arg Gly Arg Ile His Cys Cys Asp Glu Ala
 65 70 75 80
 Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
 85 90 95
 Gly Tyr His Cys Pro Gly Ile Leu Gly Leu Phe Ser Val Asp Ser Ser
 100 105 110
 Glu His Glu Ala Ile Cys Arg Gly Ser Glu Thr Lys Cys Ile Lys Ile
 115 120 125
 Ala Gly Phe Arg Arg Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile
 130 135 140
 Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
 145 150 155 160
 His Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Asp Ala
 165 170 175
 Ser Lys Ile Thr Pro Ser Glu
 180 .

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
- (B) TYPE: amino acid.
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Leu	Glu	Cys	Glu	Ile	Cys	Ile	Gly	Leu	Gly	Leu	Glu	Cys	Asn	Thr	Trp	1	5	10	15
Thr	Lys	Thr	Cys	Asp	Ala	Asn	Gln	Asp	Thr	Cys	Val	Thr	Phe	Gln	Thr	20	25	30	
Glu	Val	Ile	Arg	Ala	Pro	Val	Ser	Leu	Ser	Leu	Ile	Ser	Lys	Ser	Cys	35	40	45	
Gly	Thr	Ser	Asp	Thr	Cys	His	Leu	Asn	Tyr	Val	Glu	Thr	Ser	Pro	His	50	55	60	
Asn	Glu	Leu	Thr	Val	Lys	Thr	Lys	Arg	Thr	Cys	Cys	Thr	Gly	Glu	Glu	65	70	75	80
Cys	Lys	Thr	Leu	Pro	Pro	Pro	Val	Leu	Gly	His	Lys	Val	Asn	Pro	Pro	85	90	95	
Asn	Gly	Leu	Gln	Cys	Pro	Gly	Cys	Leu	Gly	Leu	Ser	Ser	Lys	Glu	Cys	100	105	110	
Thr	Glu	His	Leu	Val	Ser	Cys	Arg	Gly	Ser	Glu	Asn	Gln	Cys	Leu	Ser	115	120	125	
Ile	Ile	Gly	Lys	Glu	Phe	Gly	Leu	Phe	Phe	Arg	Ala	Leu	Ser	Tyr	Lys	130	135	140	
Gly	Cys	Ala	Thr	Glu	Ser	Leu	Cys	Thr	Leu	Phe	Glu	Lys	Arg	Phe	Trp	145	150	155	160
Asn	Val	Leu	Glu	Asp	Val	Glu	Val	Asp	Phe	Lys	Cys	Thr	Pro	Ala	Leu	165	170	175	
Pro	Lys	Ser	Ser	Gln												180			

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(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGA GACAGAGGAA	60
TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGATGG AGGTTTCAAC AGCACCTATT	120
TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAAC TGAACGCTTT	180
GATATAAATA TTGGACATGA TTCCTTTTTC AGAGGAAGAA TCCACTGTTG TGATGAAGCA	240
AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGAATTC TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAGAG AAAGATTTCC TGGAGACATC	420
GCTTATAATA TCAAAGGTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT	480
CACGAAGAAC ATAGAAATGA C	501

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

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CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCC AGAGTGACGA GACAGAGGAA 60

TGTGCCTCTG CAGAAGATCA ATGTGGCAGC GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAAGTGTTTC TCATCCAGCC TCTGCAAAC TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTG TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TATAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAA TAGAACTCAC 480

GAAGAACATA GAAATGATCT A 501

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGT GACAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGGTTTCAAC AGCACCTATT 120

TCCACCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAAC TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATATG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTG TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

AGTGAAACCA AATGCATTAA AATTGCGGGA TTCAGAAGAG AAAGATATCC TATAGACATC 420

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GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ATAGAAATGA T 501

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 501 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTGAGTGTG AGATTTGTAT CGGGCTGGGC CTGGAATGTA ACACCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACAGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAACTC TGCCACCGCC TGTGCTTGA CACAAAGTCA ACCCACCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCTGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAAGAAT TTGGCCTTTT CTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA A 501

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg

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1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACCTTCT TAGCTACAGG CATGTGT 57

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(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 183 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```

His Ser Cys Glu Ile Cys Arg Asn Phe Gly Lys Asp Cys Glu Ser Glu
1           5           10           15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
          20           25           30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
          35           40           45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
          50           55           60

Gly His Asp Ser Tyr Val Arg Gly Arg Ile His Cys Cys Asp Glu Glu
65           70           75           80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
          85           90           95

Gly Tyr His Cys Pro Gly Ile Leu Gly Ala Phe Ser Val Asp Ser Ser
          100          105          110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
          115          120          125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Val Asp Ile Ala Tyr Asn Ile
          130          135          140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Lys Leu Ser Asn Arg Thr
145          150          155          160

His Glu Glu Arg Arg Asn Asp Leu Ile Thr Leu Glu Cys Thr Asp Ala

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165

170

175

Ser Lys Ile Ala Pro Ser Glu

180

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 183 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Arg	Ser	Cys	Glu	Ile	Cys	His	Asn	Phe	Gly	Lys	Val	Cys	Glu	Ser	Glu
1				5					10					15	

Glu	Ala	Glu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu
		20						25					30		

Leu	Glu	Ile	Ser	Ser	Ala	Pro	Ile	Ser	Phe	Arg	Thr	Ile	His	Arg	Asn
		35					40						45		

Cys	Phe	Ser	Ser	Ser	Leu	Cys	Lys	Leu	Glu	His	Phe	Asp	Ile	Asn	Ile
	50					55						60			

Gly	His	Asp	Ser	Tyr	Ile	Arg	Gly	Arg	Ile	His	Cys	Cys	Asp	Glu	Glu
65					70					75				80	

Lys	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn
				85					90					95	

Gly	Tyr	His	Cys	Pro	Gly	Ile	Leu	Gly	Val	Phe	Ser	Val	Asp	Ser	Ser
			100					105					110		

Glu	His	Glu	Ala	Ile	Cys	Arg	Gly	Thr	Glu	Thr	Lys	Cys	Ile	Asn	Leu
		115					120					125			

Ala	Gly	Phe	Arg	Lys	Glu	Arg	Tyr	Pro	Leu	Asp	Ile	Ala	Tyr	Asn	Ile
		130					135					140			

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Ser Lys Ile Thr Pro Ser Glu
180

(i) SEQUENCE CHARACTERISTICS:

- (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu	Glu	Cys	Glu	Ile	Cys	Ile	Gly	Leu	Gly	Arg	Glu	Cys	Asn	Ser	Trp
1			5						10					15	
Thr	Lys	Thr	Cys	Asp	Ala	Asn	Gln	Asp	Thr	Cys	Val	Thr	Phe	Gln	Thr
			20					25					30		
Glu	Val	Ile	Arg	Ala	Pro	Val	Ser	Leu	Ser	Leu	Ile	Ser	Lys	Ser	Cys
		35					40					45			
Gly	Thr	Ser	Asp	Thr	Cys	His	Leu	Asn	Tyr	Val	Glu	Thr	Ser	Pro	His
	50					55					60				
Asn	Glu	Leu	Thr	Val	Lys	Thr	Lys	Arg	Thr	Cys	Cys	Thr	Gly	Glu	Glu
65					70					75					80
Cys	Lys	Thr	Leu	Pro	Pro	Pro	Val	Leu	Gly	Tyr	Lys	Val	Asn	Pro	Pro
				85					90					95	
Asn	Gly	Leu	Gln	Cys	Pro	Gly	Cys	Leu	Gly	Leu	Ser	Ser	Lys	Glu	Cys
			100					105					110		
Thr	Glu	His	Pro	Val	Ser	Cys	Arg	Gly	Ser	Glu	Asn	Gln	Cys	Leu	Ser

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115	120	125
Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys		
130	135	140
Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp		
145	150	155
Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu		
165	170	175
Pro Lys Ser Ser Gln		
180		

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACTCATGTG AAATTGTGCG CAATTTTGGG AAAGATTGTG AGAGTGAGGA GGCAGAGGAA	60
TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT	120
TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT	180
GATATAAATA TTGGACATGA TTCCTATGTG AGAGGAAGAA TCCACTGTTG TGATGAAGAA	240
AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGCATTG TTGGTGCATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACCGAGACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TGTAGACATC	420
GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAAGTTGAG CAATAGAACT	480
CACGAAGAAC GTAGAAATGA	500

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(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGCTCATGTG AAATTTGTCA CAATTTTGGG AAAGTTTGGC AGAGTGAGGA GGCAGAGGAA	60
TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTGCTGG AGATTTCATC AGCACCTATT	120
TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAAC TGAACACTTT	180
GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA	240
AAGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGCATTG TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACCGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TTTAGACATC	420
GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT	480
CACGAAGAAC ACAGAAATGA	500

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 542 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

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```

CTTGAGTGCG AGATTTGTAT TGGGCTGGGC CGGGAATGTA ACTCCTGGAC GAAAACCTGT      60
GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC      120
CTCTCTTTGA TTTCAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG      180
ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA      240
TGTAAAATC TGCCACCGCC TGTGCTTGA TACAAAGTCA ACCCACCCAA CGGACTTCAG      300
TGTCTGGAT GCCTTGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG      360
GGATCTGAAA ACCAGTGTTT GTCTATAATT GGAAGGAAT TTGGCCTTTT CTCAGAGCA      420
TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG      480
AATGTTTTAG AGGATGTTGA GACTTCAAAT GCACGCCAGC CCTCCCAAAG TCTTCCCAGT      540
GA                                                                                   542

```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1           5           10           15
Gly Ser Cys

```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Met	Lys	Ser	Leu	Gln	Ile	Ile	Cys	Leu	Leu	Phe	Val	Leu	Val	Ala	Arg
1				5				10						15	

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5				10						15	

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACCTTCT TAGCTACAGG CATGTGT

57

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 183 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Arg	Ser	Cys	Glu	Thr	Cys	His	Asn	Phe	Gly	Lys	Asp	Cys	Glu	Ser	Glu	1	5	10	15
Glu	Ala	Glu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu	20	25	30	
Leu	Glu	Ile	Ser	Ser	Ala	Pro	Ile	Ser	Phe	Arg	Ser	Ile	His	Arg	Asn	35	40	45	
Cys	Phe	Ser	Ser	Ser	Leu	Cys	Lys	Leu	Glu	His	Phe	Asp	Ile	Asn	Ile	50	55	60	
Gly	His	Asp	Ser	Tyr	Val	Arg	Gly	Arg	Ile	His	Cys	Cys	Asn	Glu	Glu	65	70	75	80
Lys	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn	85	90	95	
Gly	Tyr	His	Cys	Pro	Gly	Ile	Leu	Gly	Ala	Phe	Ser	Val	Asp	Ser	Ser	100	105	110	
Glu	His	Glu	Ala	Ile	Cys	Arg	Gly	Thr	Glu	Thr	Lys	Cys	Ile	Asn	Leu	115	120	125	
Ala	Gly	Phe	Arg	Lys	Glu	Arg	Tyr	Pro	Leu	Asp	Ile	Ala	Tyr	Asn	Ile	130	135	140	
Lys	Gly	Cys	Thr	Ser	Ser	Cys	Pro	Glu	Leu	Arg	Leu	Ser	Asn	Arg	Thr	145	150	155	160
His	Glu	Glu	His	Arg	Asn	Glu	Leu	Ile	Lys	Val	Glu	Cys	Thr	Asp	Ala	165	170	175	
Ser	Lys	Ile	Thr	Pro	Ser	Glu										180			

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 183 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

```

Arg Ser Cys Glu Ile Cys His Asn Phe Gly Lys Val Cys Glu Ser Glu
1           5           10           15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
          20           25           30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
      35           40           45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
      50           55           60

Gly His Asp Ser Tyr Ile Arg Gly Arg Ile His Cys Cys Asp Glu Glu
65           70           75           80

Lys Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
          85           90           95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
          100          105          110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
          115          120          125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Leu Asp Ile Ala Tyr Asn Ile
          130          135          140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145          150          155          160

His Glu Glu His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
          165          170          175

Ser Lys Ile Thr Pro Ser Glu

```

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180

(2). INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

```

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Arg Glu Cys Asn Ser Trp
1           5           10           15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20           25           30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35           40           45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50           55           60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65           70           75           80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Asn Pro Pro
85           90           95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100          105          110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115          120          125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130          135          140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145          150          155          160

```

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Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Ala Pro Ala Leu
 165 170 175

Pro Lys Ser Ser Gln
 180

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Leu Glu Cys Glu Phe Cys Phe Thr Pro Ala Leu Gln Cys Asp Asn Ser
 1 5 10 15

Arg Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Ser Gln
 20 25 30

Thr Glu Val Ile Arg Ala Pro Val Ser Leu Thr Phe Ile Ser Lys Ser
 35 40 45

Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro
 50 55 60

His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu
 65 70 75 80

Glu Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Asn Pro
 85 90 95

Pro Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu
 100 105 110

Cys Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu
 115 120 125

Ser Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr

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130	135	140
Lys Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe		
145	150	155 160
Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala		
	165	170 175
Leu Pro Lys Ser Ser Gln		
180		

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CGCTCATGTG AAAC TTGTCA CAATTTTGGG AAAGATTGCG AGAGTGAGGA GGCAGAGGAA	60
TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT	120
TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT	180
GATATAAATA TTGGACATGA TTCCTATGTG AGAGGAAGAA TCCACTGTTG TAATGAAGAA	240
AAGTGCGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATATCACTGC	300
CCTGGCATCC TTGGTGCATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC CTTAGACATC	420
GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT	480
CACGAAGAAC ACAGAAATGA	500

(2) INFORMATION FOR SEQ ID NO:68:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```

CGCTCATGTG AAATTTGTCA CAATTTTGGA AAAGTTGTG AGAGTGAGGA GGCAGAGGAA      60
TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT      120
TCCTTCCGAA CCATTCACAG GAACTGTTTC TCATCCAGCC TCTGCAAAC TGAACATTTT      180
GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA      240
AAGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATATCACTGC      300
CCTGGCATTG TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA      360
ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TTTAGACATC      420
GCTTATAATA TCAAAGGTTG CACTTCTTCT TGCCAGAAC TGAGGTTGAG CAATAGAACT      480
CACGAAGAAC ACAGAAATGA                                         500

```

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```

CTTGAGTGCG AGATTGTAT TGGGCTGGGC CGGGAATGTA ACTCCTGGAC GAAAACCTGT      60

```

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GATGCTAATC AAGATACTTG TGTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120
CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180
ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240
TGTAAGAACTC TGCCACCGCC TGTGCTTGA GACAAAGTCA ACCCACCCTAA CGGACTTCAG 300
TGTCTTGAT GCCTTGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG 360
GGATCTGAAA ACCAGTGTTC GTCTATAATT GGAAGGAAT TTGGCCTTTT CTTCAGAGCA 420
TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480
AATGTTTTAG AGGATGTTGA 500

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 549 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTTGAGTGTG AGTTTGTTC CACGCCAGCC CTGCAATGTG ATAACAGCAG GACGAAAACC 60
TGTGATGCTA ATCAAGATAC TTGTGTTACC TCTCAAACCTG AAGTGATAAG AGCCCTGTG 120
TCCCTCACTT TCATTTCAAA ATCCTGTGGT ACTTCTGACA CTGCCATCT TAACTACTTG 180
GAGACGAGTC CACATAATGA ACTAACGGTG AAGACCAAAA GAACCTGCTG TACTGGGGAG 240
GAATGTAAAA CTCTGCCACC GCCTGTGCTT GGAGACAAAG TCAACCCACC CAACGGACTT 300
CAGTGTCTTG GATGCCTTGG ATTGTCCTCA AAAGAATGCA CTGAACACCC GGTTCCTGC 360
CGGGGATCTG AAAACCAAGT TTTGTCTATA ATTGGGAAGG AATTTGGCCT TTTCTTCAGA 420
GCATTGTCTT ATAAAGGATG TGCTACGGAG AGTCTGTGCA CTTTATTTGA GAAGAGGTTT 480

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TGG AATGTTT TAGAGGATGT TGAAGTAGAC TTCAAATGCA CGCCAGCCCT CCCAAAGTCT 540

TCCCAGTGA 549

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Met	Lys	Ser	Leu	Gln	Ile	Ile	Cys	Leu	Leu	Phe	Val	Leu	Val	Ala	Arg
1				5				10						15	

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Met	Lys	Ser	Leu	Gln	Ile	Ile	Cys	Leu	Leu	Phe	Val	Leu	Val	Ala	Arg
1				5				10						15	

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:73:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5						10					15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5						10					15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACCTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACCTTCT TAGCTACAGG CATGTGT

57

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 183 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Arg	Ser	Cys	Glu	Ile	Cys	His	Asn	Phe	Gly	Lys	Val	Cys	Asp	Asn	Glu
1				5					10					15	

Pro	Ala	Leu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu
			20					25					30		

Leu	Glu	Ile	Ser	Ser	Ala	Pro	Ile	Ser	Phe	Arg	Thr	Ile	His	Arg	Asn
	35						40					45			

Cys	Phe	Ser	Ser	Ser	Leu	Cys	Lys	Leu	Glu	His	Phe	Asp	Ile	Asn	Ile
	50					55					60				

Gly	His	Asp	Ser	Tyr	Ile	Arg	Gly	Arg	Ile	His	Cys	Cys	Asp	Glu	Glu
65					70				75					80	

Lys	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn
				85					90					95	

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Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
 100 105 110
 Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
 115 120 125
 Ala Gly Phe Arg Lys Glu Arg Thr Pro Leu Asp Ile Ala Tyr Asn Ile
 130 135 140
 Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
 145 150 155 160
 His Gly Gly His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
 165 170 175
 Pro Lys Ile Thr Pro Ser Glu
 180

(2) INFORMATION FOR SEQ ID NO:80:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 181 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Leu Glu Cys Asp Ile Cys Ile Gly Leu Gly Arg Glu Cys Asn Thr Trp
 1 5 10 15
 Thr Lys Thr Cys Asp Ala Asn Gln Asp Ala Cys Val Thr Phe Gln Thr
 20 25 30
 Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
 35 40 45
 Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro His
 50 55 60
 Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu

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65 70 75 80
 Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Ser Pro Pro
 85 90 95
 Asn Gly Leu Gln Cys Pro Gly Cys Phe Gly Leu Ser Ser Lys Glu Cys
 100 105 110
 Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
 115 120 125
 Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
 130 135 140
 Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Lys Phe Trp
 145 150 155 160
 Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
 165 170 175
 Pro Lys Ser Ser Gln
 180

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Leu Glu Cys Asp Ile Cys Phe Gly Leu Gly Arg Lys Cys Asn Thr Trp
 1 5 10 15
 Thr Lys Thr Cys Asp Ala Asn Gln Asp Ala Cys Val Thr Phe Gln Thr
 20 25 30
 Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
 35 40 45

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Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro His
 50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
 65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Ser Pro Pro
 85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Phe Gly Leu Ser Ser Lys Glu Cys
 100 105 110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
 115 120 125

Leu Ile Gly Lys Glu Phe Gly Phe Phe Phe Arg Ala Leu Ser Tyr Lys
 130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Lys Phe Trp
 145 150 155 160

Asn Val Leu Glu Glu Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
 165 170 175

Pro Lys Ser Ser Gln
 180

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CGCTCATGTG AAATTTGTCA CAATTTTGGG AAAGTTTGCG ACAATGAGCC GGCATTGGAA 60
 TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC GGCACCTATT 120

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TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT	180
GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA	240
AAGTGGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC	300
CCTGGCATTG TTGGTGATT CTCACTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA	360
ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGAACTCC TTTAGACATC	420
GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT	480
CACGGAGGAC ATAGAAATGA	500

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 500 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CTTGAGTGTG ATATTTGTAT TGGGCTGGGC CGGGAATGTA ACACCTGGAC GAAAACCTGT	60
GACGCTAATC AAGATGCTTG TGTACCTTT CAACTGAAG TGATAAGAGC CCCTGTGTCC	120
CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACCTGGAG	180
ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA	240
TGTAAAACTC TGCCACCGCC TGTGCTTGA GACAAAGTCA GCCCACCAA CGGACTTCAG	300
TGTCCTGGAT GCTTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG	360
GGATCTGAAA ACCAGTGCTT GTCCATAATT GGGAAGGAAT TTGGCCTTTT CTCAGAGCA	420
TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAAGTTCTGG	480
AATGTTTTAG AGGATGTTGA	500

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(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CTTGAGTGTG ATATTGTTT TGGGCTGGGC CGGAAATGTA ACACCTGGAC GAAAACCTGT	60
GATGCTAATC AAGATGCTTG TGTACTTTT CAACTGAAG TGATAAGAGC CCCTGTGTCC	120
CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACCTGGAG	180
ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA	240
TGTAAAACTC TGCCACCGCC TGTGCTTGA GACAAAGTCA GCCCACCCAA CGGACTTCAG	300
TGTCCTGGAT GCTTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG	360
GGATCTGAAA ACCAGTGTCT GTCTCTAATT GGGAAGGAAT TTGGCTTTTT CTTCAGAGCA	420
TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTC TATTTGAGAA GAAGTTCTGG	480
AATGTTTTAG AGGAAGTTGA AGTAGACTTC AAATGCACCC CAGCCCTCCC AAAGTCTTCC	540
CAGTGA	546

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Met	Lys	Ser	Leu	Gln	Ile	Ile	Cys	Leu	Leu	Phe	Val	Leu	Val	Ala	Arg
1				5					10					15	

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5					10					15	

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5					10					15	

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Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTGG TAGCCAGAGG AAGCTGT

57

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCATTCTCT TAGCTACAGG CATGTGT

57

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACCTTCT TAGCCACAGG CATGTGT

57

CLAIMS:

1. An isolated molecule capable of inhibiting two or more types of phospholipase enzymes.
2. The isolated molecule according to claim 1 wherein the phospholipase enzyme is a PLA₂.
3. The isolated molecule according to claim 2 wherein the types of phospholipase enzymes are PLA₂ Type I, Type II and/or Type III.
4. The isolated molecule according to any one of claims 1 to 3 derivable from the serum of a venomous animal.
5. The isolated molecule according to claim 4 wherein the venomous animal is a snake.
6. The isolated molecule according to claim 5 wherein the snake is selected from *Notechis scutatus*, *Notechis ater*, *Oxyuranus scutellatus*, *Oxyuranus microlepidotus* and *Pseudonaja textilis* or a relative thereof.
7. The isolated molecule according to claim 6 in sequencably pure and/or substantially homogeneous form.
8. The molecule according to claim 7 when associated with a carrier molecule.
9. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 1-4 or 9-12 or an amino acid sequence having at least about 40% similarity thereto.
10. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 17-38 or 43-45 or an amino acid sequence having at least

about 40% similarity thereto.

11. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 51-53 or 57-59 or an amino acid sequence having at least about 40% similarity thereto.

12. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 63-66 or 71-74 or an amino acid sequence having at least about 40% similarity thereto.

13. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 79-81 or 85-87 or an amino acid sequence having at least about 40% similarity thereto.

14. A PLA_2 inhibitor $\alpha_m\beta_n$ wherein α is an α -chain of a PLA_2 inhibitor; β is a β -chain of a PLA_2 inhibitor; m is an integer from 0 to 10; n is an integer from 0 to 10 with proviso that if m and n are not 0, then $m > n$ and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

15. The isolated molecule according to claim 1 when used to inhibit a phospholipase enzyme.

16. A composition useful for the inhibition of phospholipase enzyme activity comprising a molecule according to any one of claims 1 to 14 and a pharmaceutically acceptable carrier and/or diluent.

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17. An isolated nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide capable of inhibiting two or more types of phospholipase enzymes.
18. An isolated nucleic acid molecule according to claim 17 wherein the phospholipase enzyme is a PLA₂.
19. An isolated nucleic acid molecule according to claim 18 wherein the types of phospholipase enzymes are PLA₂ Type I, Type II and/or Type III.
20. An isolated nucleic acid molecule according to claim 18 or 19 wherein the PLA₂ is derivable from the serum of a venomous animal.
21. An isolated nucleic acid molecule according to claim 20 wherein the venomous animal is a snake.
22. An isolated nucleic acid molecule according to claim 21 wherein the snake is selected from *Notechis scutatus*, *Notechis ater*, *Oxyuranus scutellatus*, *Oxyuranus microlepidotus* and *Pseudonaja textilis* or a relative thereof.
23. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 1-4 or 9-12 or an amino acid sequence having at least about 40% similarity thereto.
24. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 17-38 or 43-45 or an amino acid sequence having at least about 40% similarity thereto.
25. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 51-53 or 57-59 or an amino acid sequence having at least about 40% similarity thereto.

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26. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 63-66 or 71-74 or an amino acid sequence having at least about 40% similarity thereto.
27. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 79-81 or 85-87 or an amino acid sequence having at least about 40% similarity thereto.
28. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 5-8 or 13-16 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.
29. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 39-42 or 47-50 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.
30. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 54-56 or 60-62 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.
31. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 67-70 or 75-78 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.
32. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 82-84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more

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sequences under low stringency conditions at 42°C.

33. A nucleic acid molecule encoding a PLA₂ inhibitor having the structure:



wherein

α is an α -chain of a PLA₂ inhibitor;

β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

34. A nucleic acid molecule encoding a PLA₂ inhibitor having the structure:



wherein

α is an α -chain of a PLA₂ inhibitor;

β is a β -chain of a PLA₂ inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by a nucleotide sequence selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and 88 or a nucleotide sequence having at least about 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide

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sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C.

35. A method of treatment of the phospholipase-related symptom(s) of rheumatoid arthritis, osteoarthritis, asthma, allergic reaction, psoriasis, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome or the toxic effects of toxins in a human or animal subject, said method comprising administering an isolated molecule according to any one of claims 1 to 14 to a subject for a time and under conditions sufficient to partially or completely inhibit the activity of a phospholipase enzyme producing said symptom(s).

36. A method of isolating a PLA₂ inhibitor protein from snake blood, serum or other blood product at least comprising the steps of:

- (i) preparing a serum sample from clotted blood; and
- (ii) subjecting the serum to ion-exchange chromatography.

37. The method according to paragraph 36 wherein the PLA₂ inhibitor protein is selected from NSI, NAI, OSI, OMI and PTI.

38. An isolated polypeptide capable of inhibiting two or more of PLA₂ Type I, II and/or III wherein said polypeptide has an alpha chain comprising the following amino acid sequence:

Xaa Ser Cys Glu Xaa Cys Xaa Asn Xaa Gly Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Glu
Cys Ala Ser Xaa Glu Asp Gln Cys Gly Thr Val Leu Xaa Glu Xaa Ser Xaa Ala Pro Ile Ser
Xaa Arg Xaa Ile His Arg Xaa Cys Phe Ser Ser Ser Xaa Cys Lys Leu Glu Xaa Phe Asp Ile
Asn Ile Gly His Asp Ser Xaa Xaa Arg Gly Arg Ile His Cys Cys Xaa Glu Xaa Xaa Cys Glu
Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Xaa Gly
Xaa Phe Ser Val Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Xaa Glu Thr Lys Cys Ile Xaa
Xaa Ala Gly Phe Arg Xaa Glu Arg Xaa Xaa Xaa Asp Xaa Xaa Tyr Asn Ile Lys Gly Cys Thr
Ser Ser Cys Pro Glu Leu Xaa Leu Xaa Asn Arg Thr His Xaa Xaa Xaa Xaa Asn Xaa Leu Ile

Xaa Xaa Glu Cys Thr Xaa Ala Xaa Lys Xaa Xaa Pro Ser Glu.

39. An isolated polypeptide of claim 38 encoded by the following nucleotide sequence:

CNCTCATGTGAAANTTGTNCNAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTCA
NCNGCACCTATTTCCNNCCGANCCATT CANAGGAANTGTTTCTCATCCAGCNTCT
GCAAAC TNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNAATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATT CAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

40. An isolated nucleic acid molecule comprising the nucleotide sequence:

CNCTCATGTGAAANTTGTNCNAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTCA
NCNGCACCTATTTCCNNCCGANCCATT CANAGGAANTGTTTCTCATCCAGCNTCT
GCAAAC TNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNAATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATT CAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

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41. An isolated polypeptide capable of inhibiting two or more of PLA₂ Type I, II and/or III wherein said polypeptide has a beta chain comprising the following amino acid sequence:

Leu Glu Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Asn Xaa Xaa Thr Lys Thr
Cys Asp Ala Asn Gln Asp Xaa Cys Val Thr Xaa Gln Thr Glu Val Ile Arg Ala Pro Val Ser
Leu Xaa Xaa Ile Ser Lys Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Xaa Glu Thr
Ser Pro His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu Cys Lys
Thr Leu Pro Pro Pro Val Leu Gly Xaa Lys Val Xaa Pro Pro Asn Gly Leu Gln Cys Pro Gly
Cys Xaa Gly Leu Ser Ser Lys Glu Cys Thr Glu His Xaa Val Ser Cys Arg Gly Ser Glu Asn
Gln Cys Leu Ser Xaa Ile Gly Lys Glu Phe Gly Xaa Phe Phe Arg Ala Leu Ser Tyr Lys Gly
Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Xaa Phe Trp Asn Val Leu Glu Xaa Val
Glu Val Asp Phe Lys Cys Xaa Pro Ala Leu Pro Lys Ser Ser Gln.

42. An isolated polypeptide of claim 38 encoded by the following nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAACCTGAAGTGATA
AGAGCCCCCTGTGTCCCTCNCTTTNATNTCAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACCTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCACCCAACGGACTTCAGTGTCTGATGCNTTGGATTGT
CCTCAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNNTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

43. An isolated nucleic acid molecule comprising the nucleotide sequence:

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CTTGAGTGNGANNTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAAGTGAAGTGATA
AGAGCCCCCTGTGTCCCTCNCCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCACCCAACGGACTTCAGTGTCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

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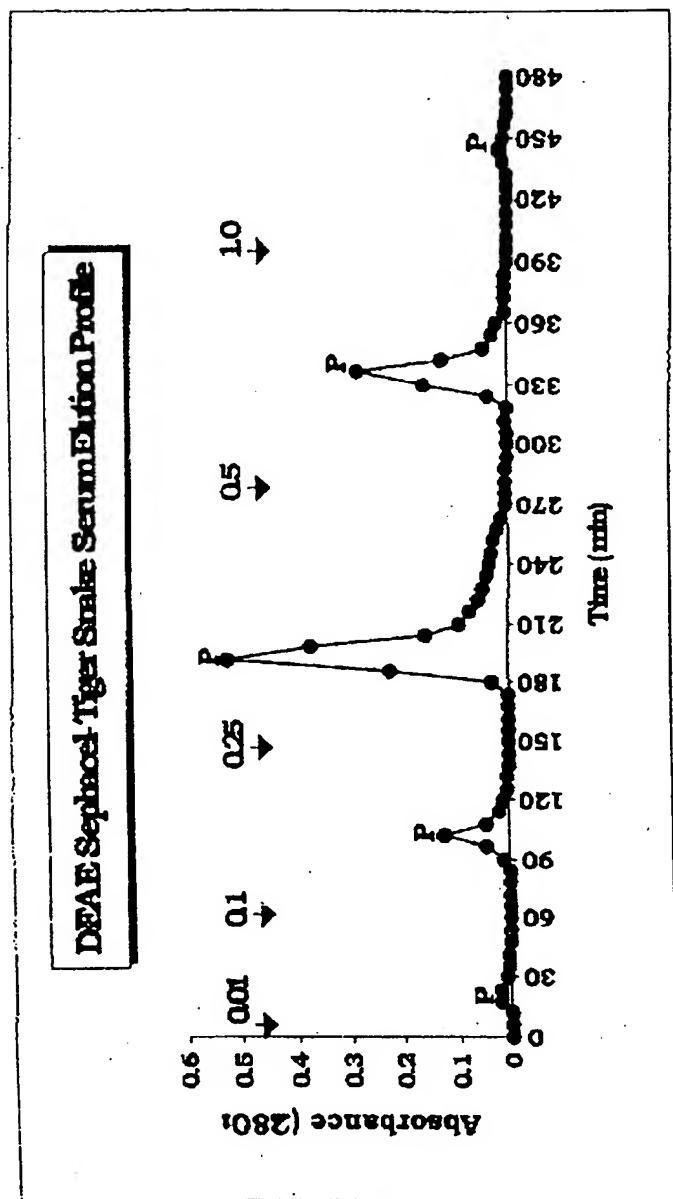


FIGURE 1A

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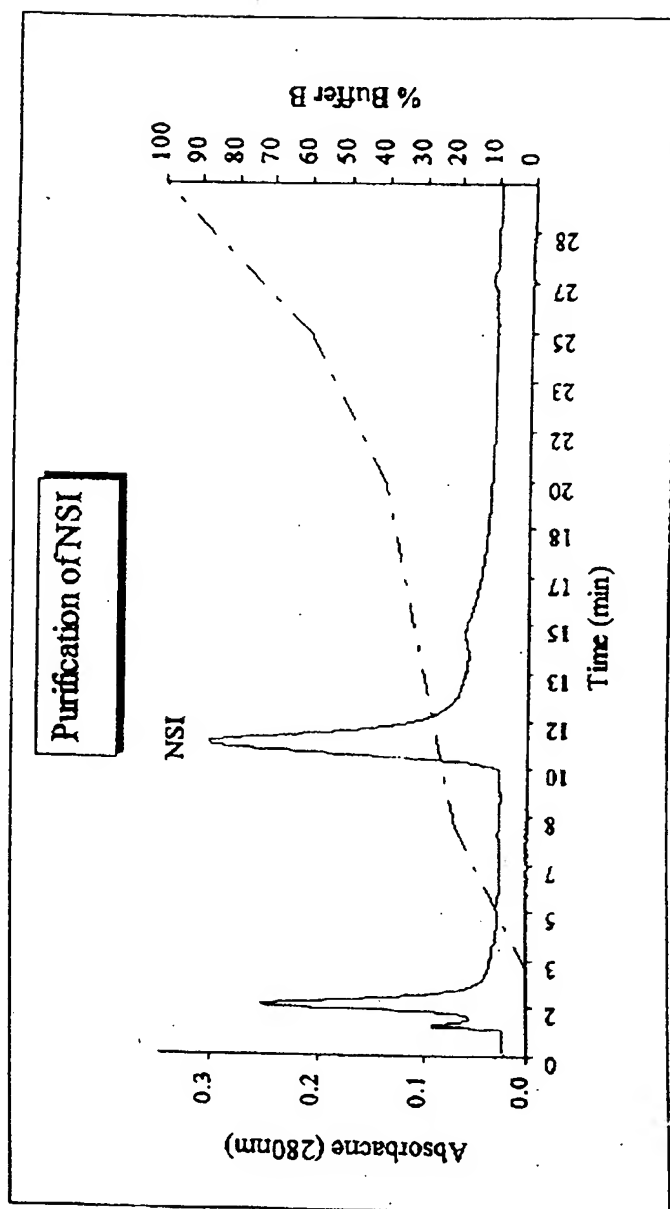


FIGURE 1B

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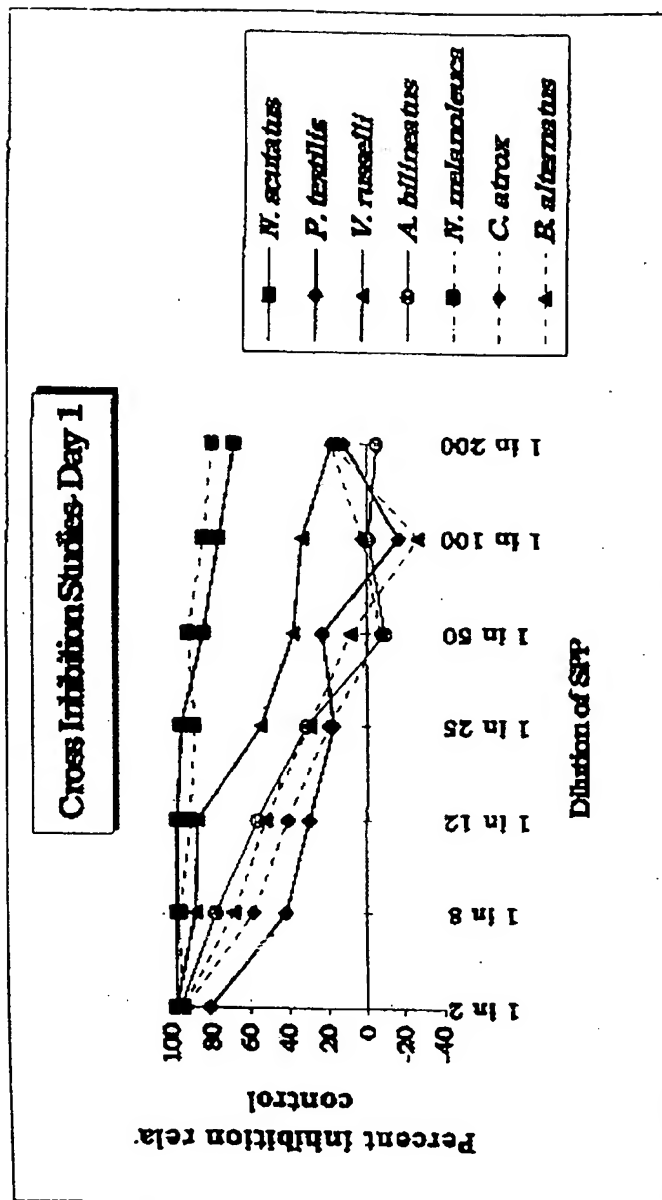


FIGURE 2A

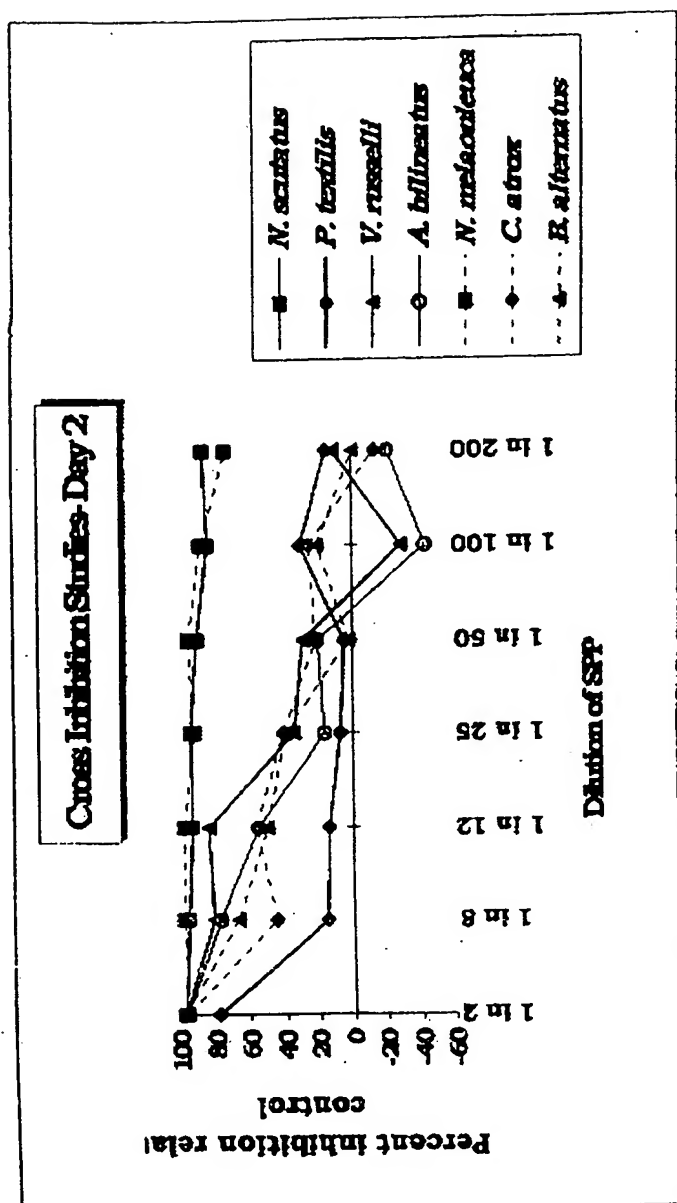


FIGURE 2B

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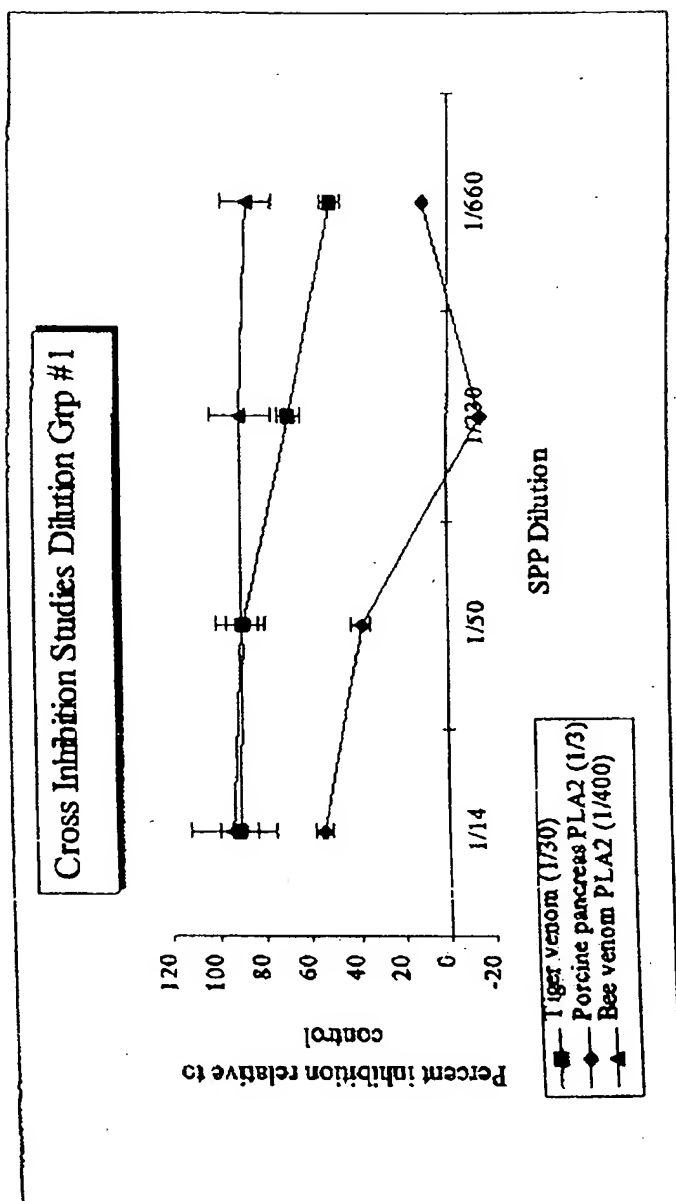


FIGURE 3A

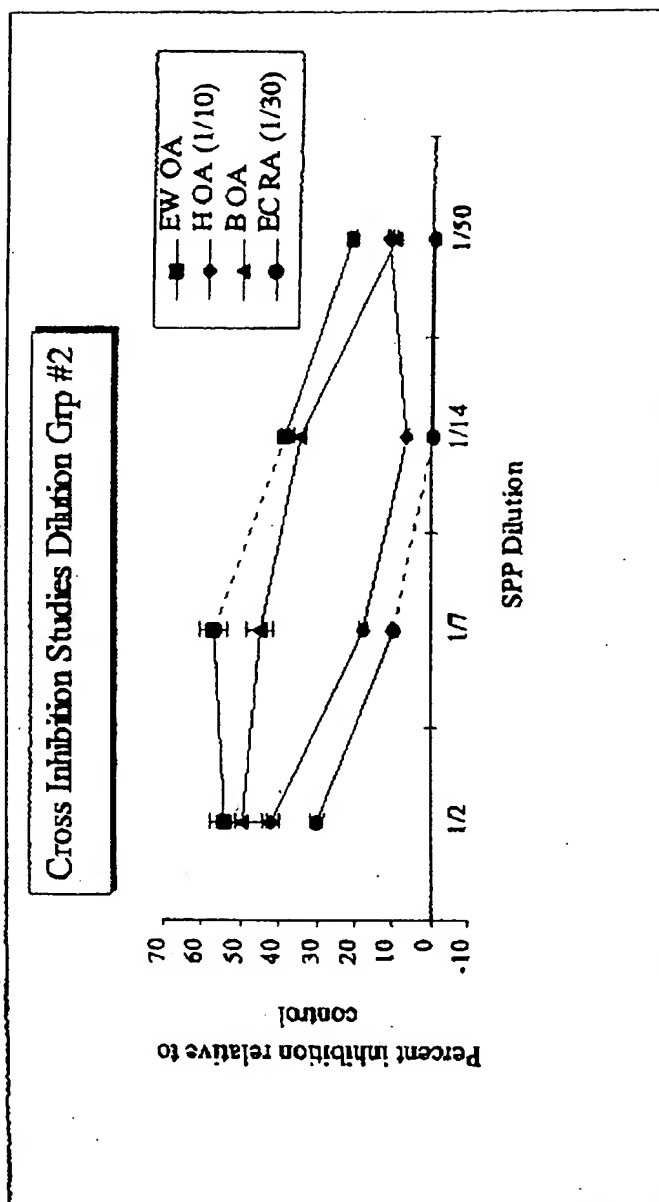


FIGURE 3B

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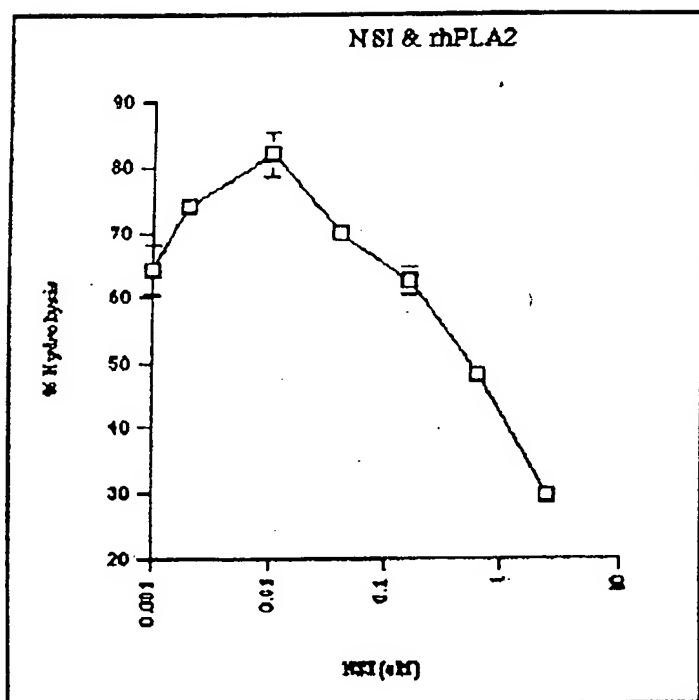


FIGURE 4

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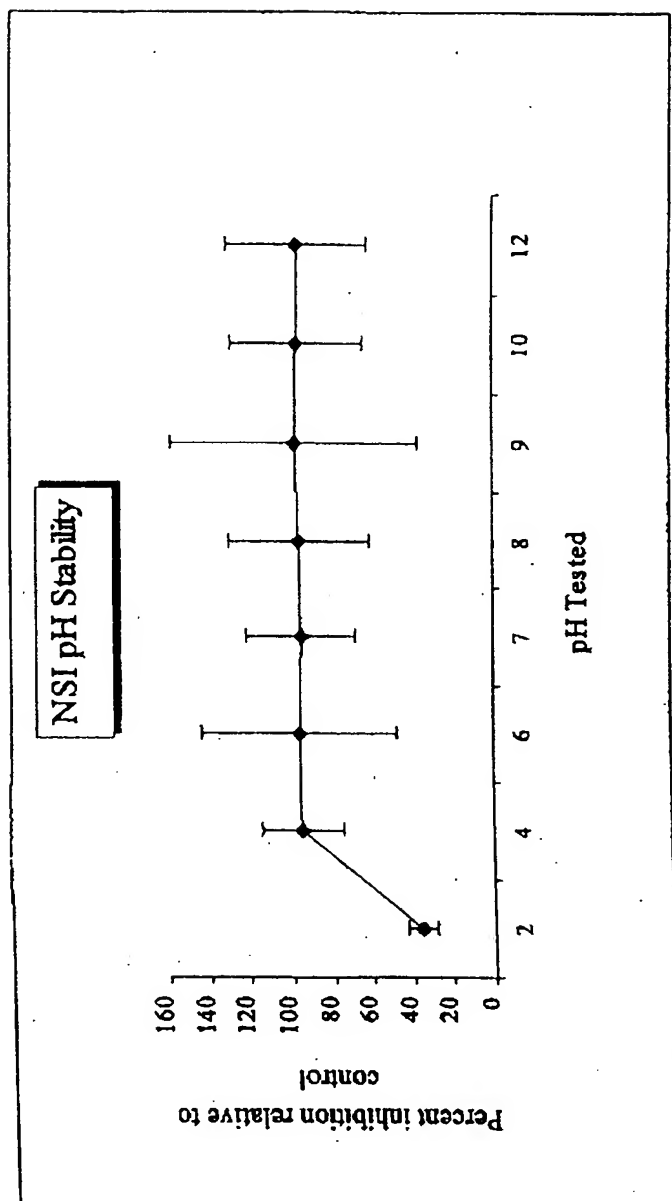


FIGURE 5A

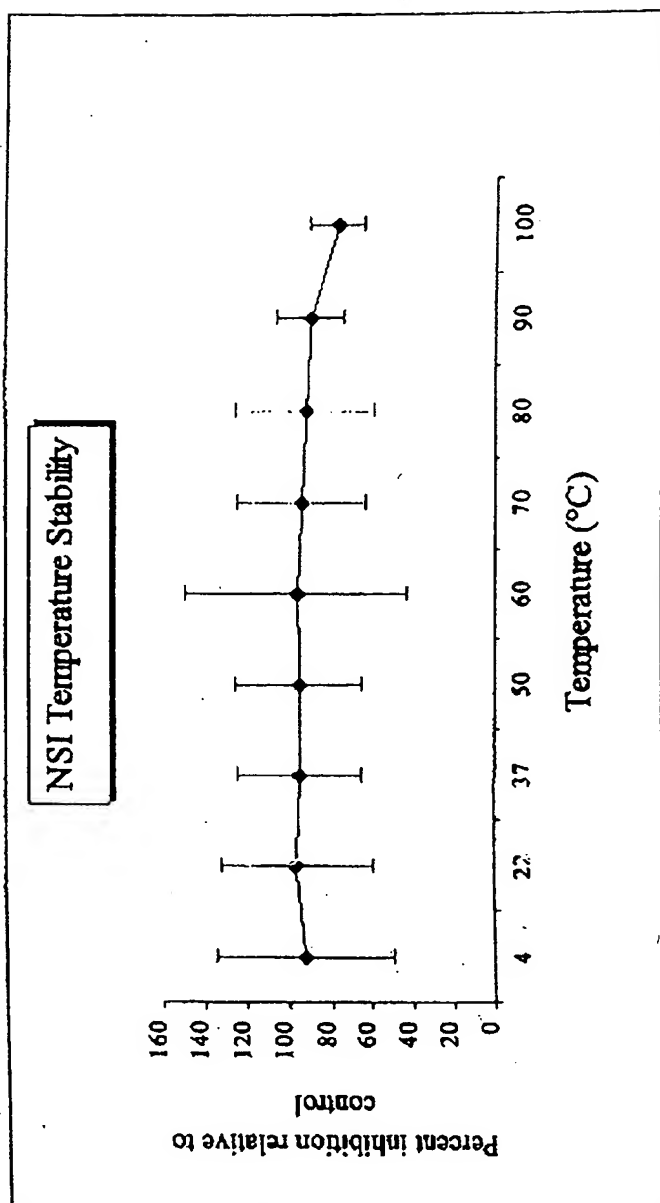


FIGURE 5B

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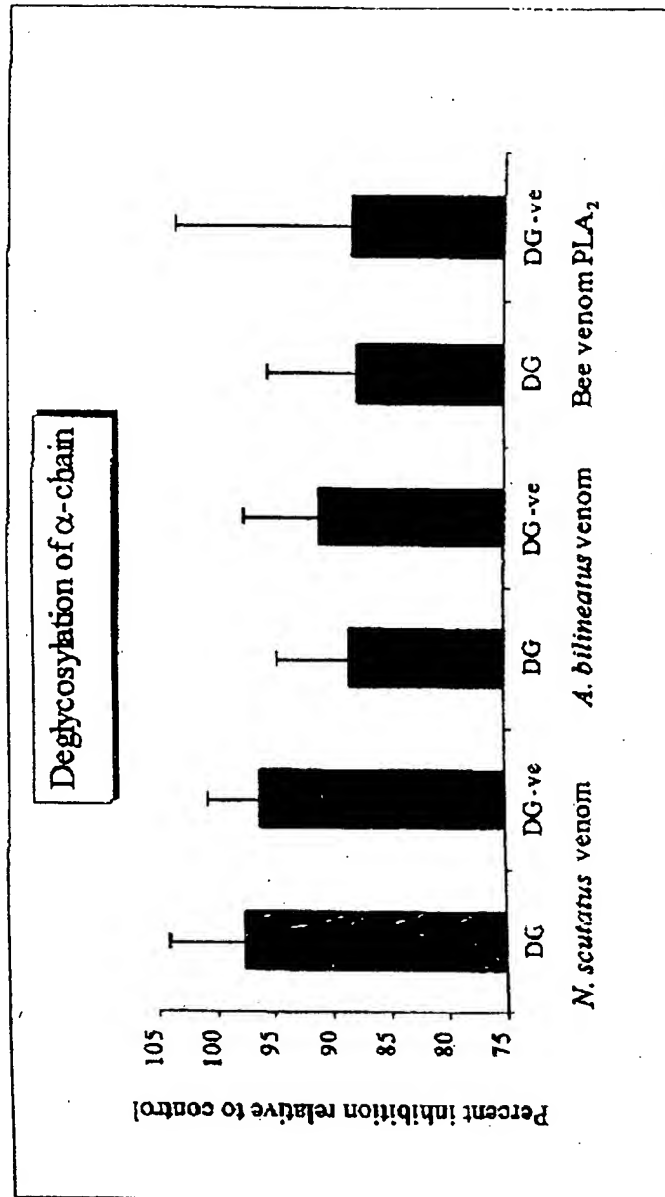


FIGURE 6A

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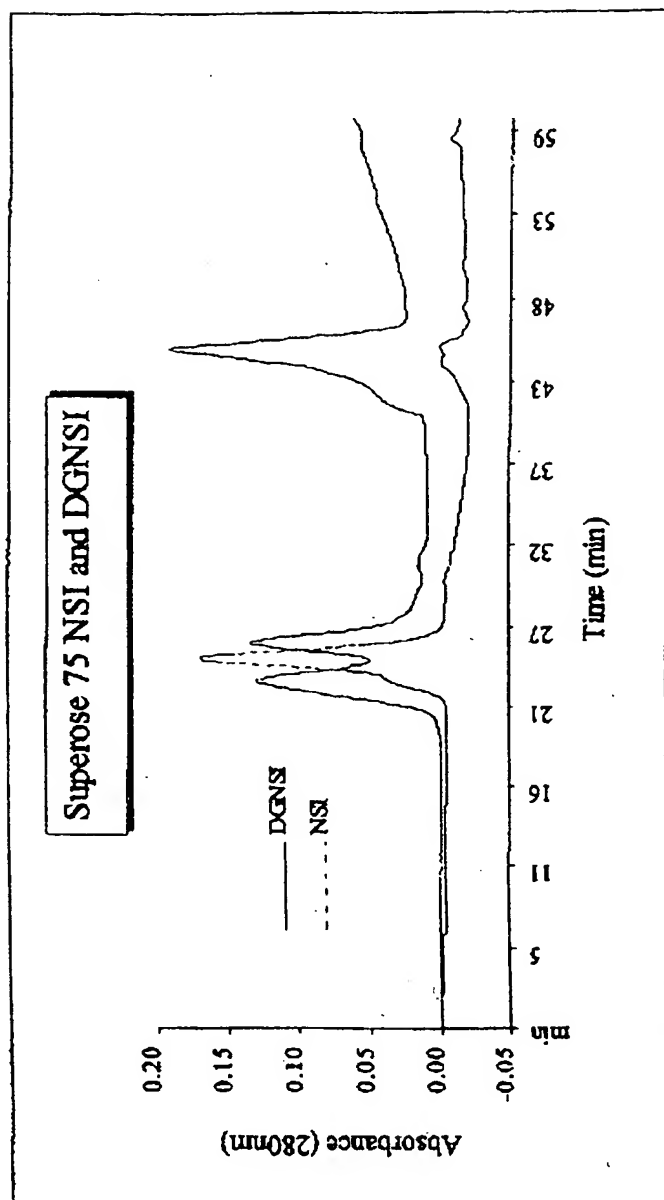


FIGURE 6B

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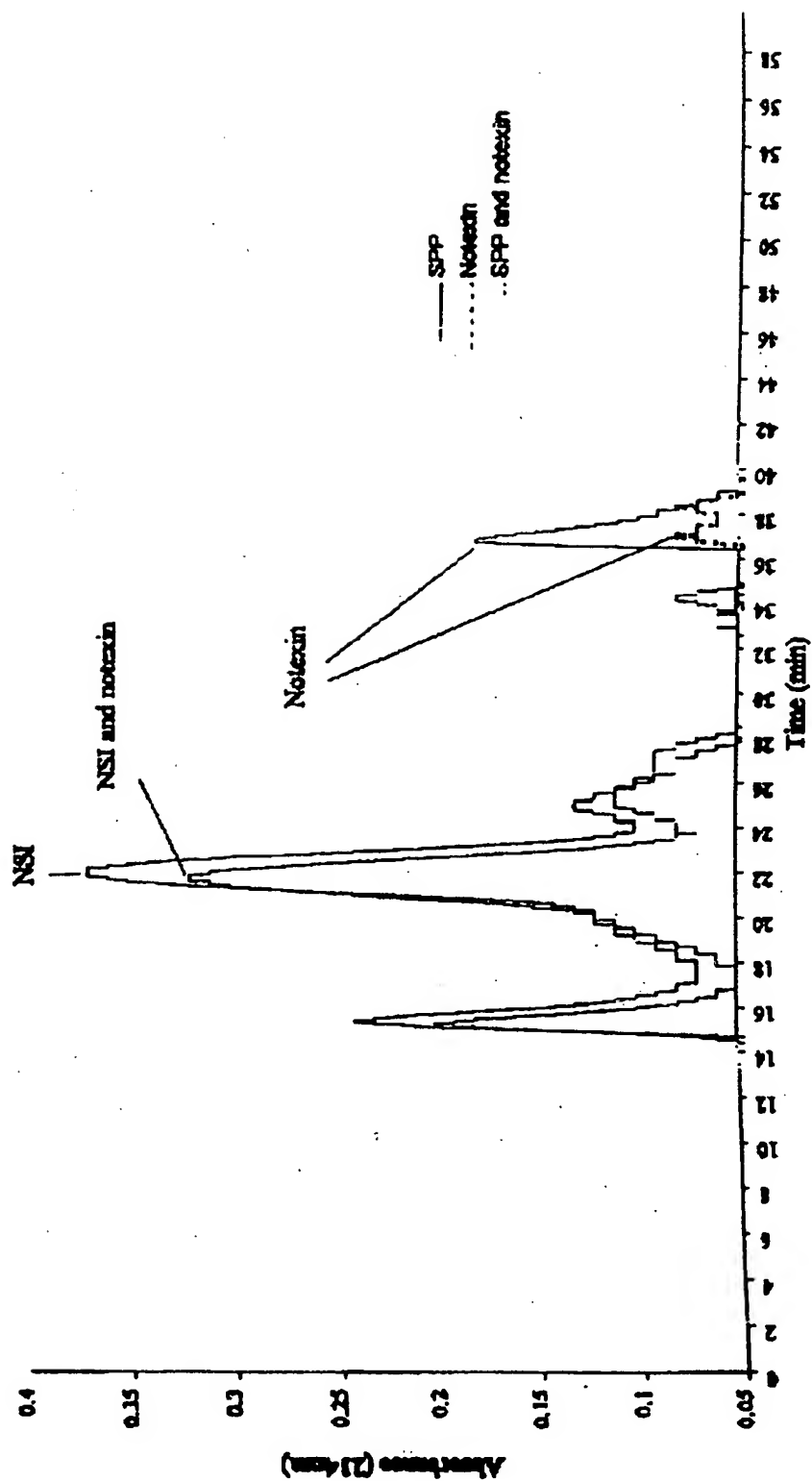


FIGURE 7

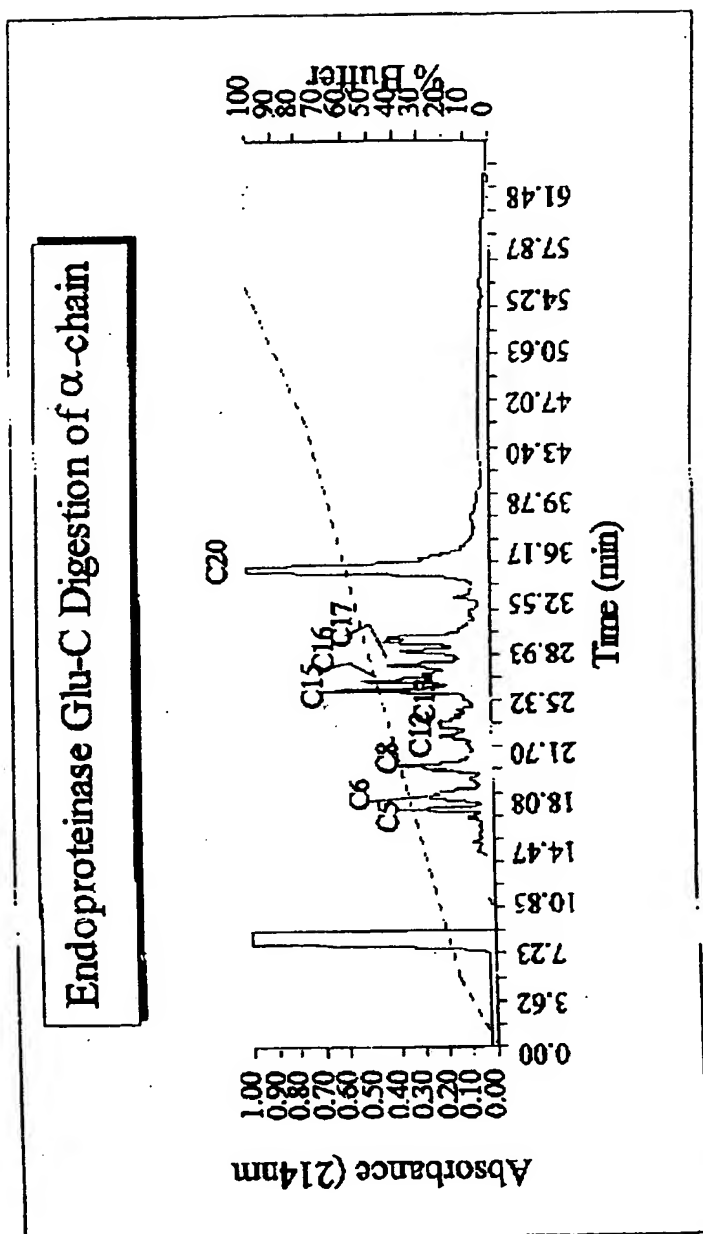


FIGURE 8

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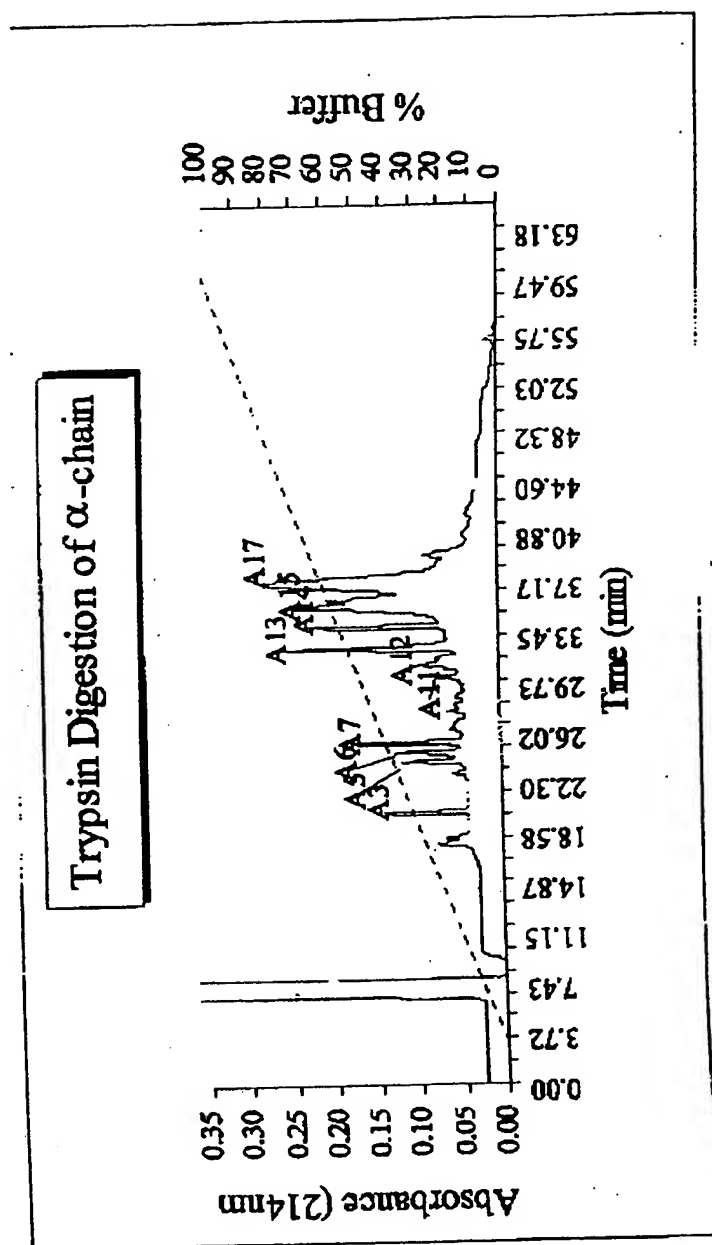


FIGURE 9

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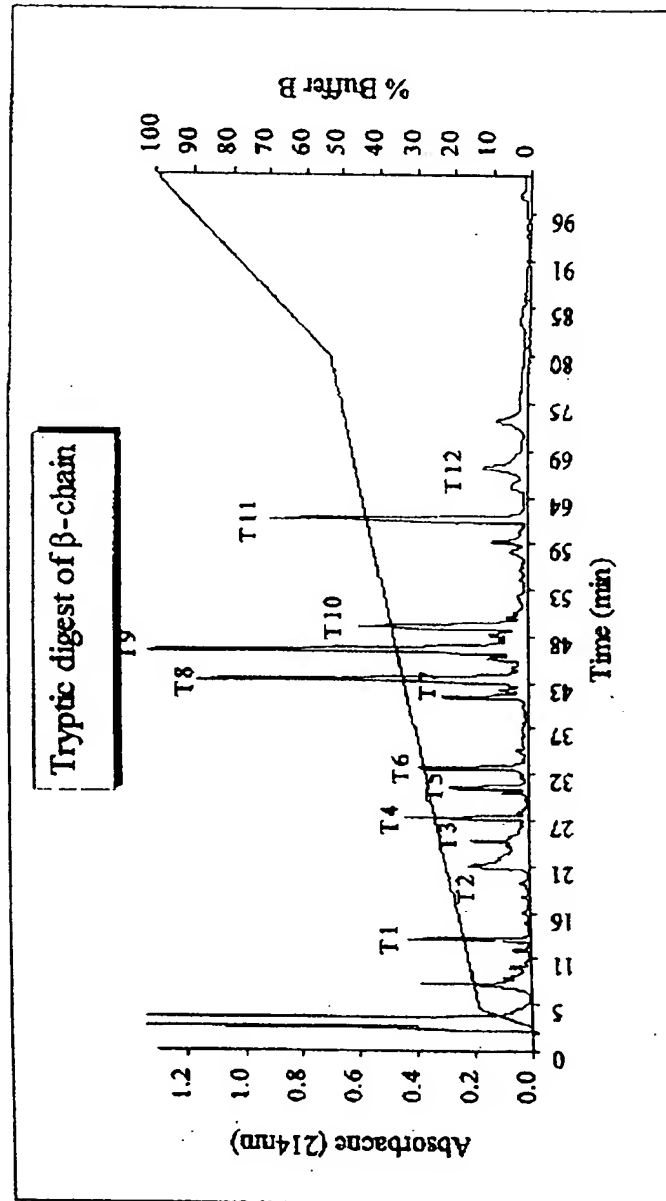


FIGURE 10

proseqnsil MKSLQIICLLFVLVARGSCHSCEICHNLGRDCETEEAEEECASPEDQCGTVLMEVSSAPIS 60
pseqctl MISLQIICFLFVLVARGSCHSCEICRNFGKDCESSEEAEEECASPEDQCGTVLLEISSAPIS 60
pseqitl MKSLQIICPLFVLVARGSCRSCEICHNFGKDCESSEEAEEECASPEDQCGTVLLEISSAPIS 60

proseqnsil FRSIHRNCFSSSLCKLERFDINIGHDSYLRGRIHCCDEARCEAAQFPGLPLSFPNGYHCP 120
pseqctl FRSIHRNCFSSSLCKLEHFDINIGHDSYVRGRIHCCDEERCEAAQFPGLPPLSFPNGYHCP 120
pseqitl FRSIHRNCFSSSLCKLEHFDINIGHDSYVRGRIHCCDEERCEAAQFPGLPLSFPNGYHCP 120

FIGURE 11 (CONT.I)

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proseqnsi1 GILGVFSVDSSEHEAICRGTTETKCINLAGFRKERFPGDIAYNIKGCTSSCPRLSLNRAH 180
pseqctl1 GILGAFSVDSSEHEAICRGTTETKCINLAGFRKERYPVDIAYNITGCTSSCPKLKLSNRTH 180
pseqitl1 GILGAFSVDSSEHEAICRGTTETKCINLAGFRKERYPVDIAYNIKGCTSSCPKLKLSNRTH 180

proseqnsi1 EEDRNDLIKVECTDASKITPSEI 203
pseqctl1 AERRNALITLDCDASKIAPSE- 202
pseqitl1 EERRNDLITLECTDASKITPSE- 202

FIGURE 11 (CONT. II)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00992

A. CLASSIFICATION OF SUBJECT MATTER		
Int Cl ⁶ : C07K 14/46, 19/00; C07H 21/04; A61K 38/17		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN, FILE REG, SUBSEQUENCE SEARCH; ANGIS, BLAST P, FAST A, SEQUENCE SEARCH		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98/13376 A, (GARVAN INSTITUTE OF MEDICAL RESEARCH), 2 April 1998. See whole document	1-3, 10, 15 16, 35
X	Biochem.Biophys. Res. Commun., 204, 1212-1218, (1994), N. Ohkura <i>et al.</i> , "The two subunits of a Phospholipase A ₂ Inhibitor from the Plasma of Thailand Cobra..."	1-5, 7-13 15, 16, 35
X	Eur. J. Biochem., 249, 838-845 (1997), I. Nobuhisa <i>et al.</i> , "Characterisation and evolution of a gene encoding a <i>Trimeresurus flavoviridis</i> serum protein..."	1-5, 7-13 15-32, 35, 36
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
Date of the actual completion of the international search 23 December 1998		Date of mailing of the international search report 21 JAN 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer L.F. McCAFFERY Telephone No.: (02) 6283 2573

INTERNATIONAL SEARCH REPORT

		international application No. PCT/AU 98/00992
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hoppe-Seyler's Z. Physiol. Chem., <u>360</u> , 1075-1090, (1979), F. J. Joubert <i>et al.</i> , "The Amino Acid Sequence of the Subunits of two reduced and S-carboxymethylated proteins..."	1-3, 7-13 15, 16, 35
X	J. Biol. Chem., 269(22), 15646-15651, (1994), C. L. Fortes-Dias <i>et al.</i> , "A Phospholipase A ₂ Inhibitor from the Plasma of the South American Rattlesnake (<i>Crotalus durissus terrificus</i>)."	1-5, 7-13 15-32, 35, 36
X	Medline Abstract PMID 7851385, Eur. J. Biochem., <u>227</u> , 19-26, (1995), J. Perales <i>et al.</i> , "Molecular Structure and Mechanism of action of the crotoxin inhibitor from <i>Crotalus durissus terrificus</i> serum."	1-5, 7, 8, 10 15, 16, 35, 36

INTERNATIONAL SEARCH REPORT

international application No.

PCT/AU 98/00992

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-8, 17-22, 36

because they relate to subject matter not required to be searched by this Authority, namely:

These claims are drafted in such a way as to make a complete search impossible on economic grounds. Accordingly the search has been substantially limited to sequences disclosed in the specification.

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00992

Information on patent family members

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	98/13376	AU	43712/97
END OF ANNEX			